STUDIES IN COUMARINS FROM SOME MEMBERS OF UMBELLIFERAE

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A C K N O W L E D G E M E N T

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A B S T R A C T

1. From the petroleum ether extract of Heracleum candicans furocoumarins, heraclenin, 8-geranoxypsoralen and a mixture of 8-geranoxypsoralen and imperatorin were isolated.
2. Benzene extract of H.candicans yielded heraclenol, the dihydroxy derivative of heraclenin.
3. The structures of heraclenin and heraclenol as 8-(β,γ-oxido-isoamyloxy)-psoralen and 8-(β,γ-dihydroxy-isoamyloxy)-psoralen respectively have been established on the basis of degradation and spectrometric studies.
4. Osthol and an ester of p-hydroxy cinnamic acid and fenchyl alcohol were isolated from the roots of Seseli sibiricum.
5. From Angelica glauca Edgw, isoimperatorin, prangolarin and a new furocoumarin of the molecular formula $C_{17}H_{18}O_6$ have been isolated. A tentative structure on the basis of spectrometric studies has been proposed for the new compound.

C O N T E N T S

I N T R O D U C T I O N

INTRODUCTION

The work described in this thesis was carried out under a scheme for the investigation of indigenous medicinal plants. Though coumarins have been known to possess toxic properties, systematic investigation of their varied physiological action, began with the isolation of psoralen type of compounds from the Egyptian plant Ammi majus, used since ancient times in the treatment of leucoderma. Since then the skin photosensitizing action and the anticoagulant properties of coumarins have been studied thoroughly both in India and abroad and the structure-activity relationship has been reviewed by several workers.

Various plants belonging to the family Umbelliferae, known to be a rich source of this class of compounds, were investigated and their coumarin constituents characterised. These included Heracleum candicans, Seseli sibiricum and Angelica glauca. From the first, the furocoumarin heraclenin (LXXIV) was isolated and its structure was shown to be similar to that of oxypeucedanin and prangolarin, from which it differs only in the position of the side chain. Apart from heraclenin, heraclenol (LXXVI) the dihydroxy derivative of heraclenin, 8-geranoxypsoralen (LXXXI) and a mixture of 8-geranoxypsoralen and imperatorin (LXXX) were isolated.

8-Geranoxypsoralen (LXXXI) has previously been reported from Citrus oil but its structure was not conclusively proved. The isolation of a complex mixture of imperatorin and 8-geranoxypsoralen, which at first appeared to be the geometrical isomer of 8-geranoxypsoralen, made a reinvestigation of the nature of the side chain necessary.

From Seseli sibiricum osthol and a derivative of fenchyl alcohol and p-hydroxycinnamic acid (LXXXVIII) were obtained and characterised. The essential oil constituents of this plant have already been analysed by earlier workers.

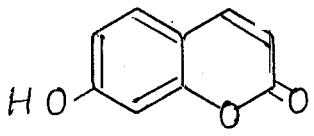
Angelica glauca contains a number of coumarins, from which prangolarin (XC) and isoimperatorin (LXXXIX) were separated and characterised. Apart from these two, a small quantity of a third coumarin was isolated and a tentative structure, based on spectrometric studies, is proposed.

Preliminary pharmacological investigation of heraclenin (LXXIV) has shown it to possess better anticoagulant properties than sintrom.

THEORETICAL

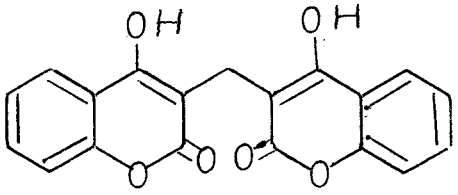
A systematic investigation of naturally occurring coumarins was first undertaken by Spath in 1930s. The chemistry of a large number of substituted coumarins and furocoumarins was studied, and various methods of degradation were introduced by him. These included degradation of furocoumarins to furan-2,3-dicarboxylic acid[1], and cleavage of the isoprenoxy side chain, either by sublimation in vacuum, or by heating with acetic acid-sulphuric acid mixture[2].

At present about one hundred naturally occuring coumarins are described in literature. All but six of these were isolated from plants, and the rest were isolated from animals or micro-organisms. In particular plants belonging to the natural order Orchidaceae, Leguminoseae, Rutaceae, Umbelliferae and Labiatae are rich sources of this class of compounds. Several coumarin glycosides have been found in nature but they are not as widely distributed as glycosides of some other related compounds such as flavones. Glycosides of the corresponding o-hydroxy cinnamic acids[3] are also known, with the o-hydroxy group blocked by combination with a sugar moiety. Umbelliferone (I) can be regarded



I

as the precursor of most coumarins, as with few exceptions coumarins are substituted in the 7-position. Of the six theoretically possible hydroxy coumarins, 3-hydroxy coumarin has not so far been found in nature, and 4-hydroxy derivatives are very rare e.g. dicoumarol (II),ammoresinol (III), ferulenol (IV).



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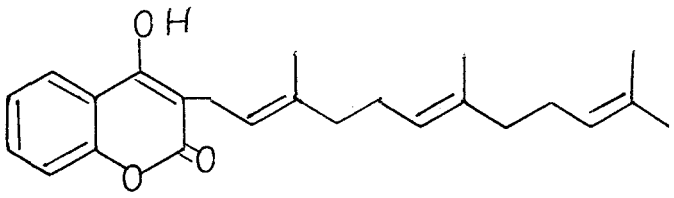

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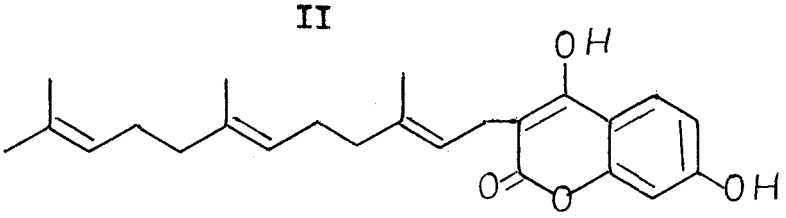
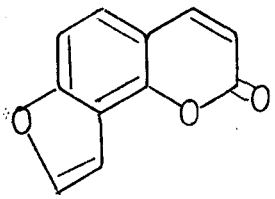


IV

Frequently the oxygen atom at 7-position undergoes cyclisation to give rise to angular or linear furocoumarins or pyranocoumarins, such as isopsoralen (V), psoralen (VI) and xanthyletin (VII). There are only three reported examples of coumarins where another oxygen atom participates



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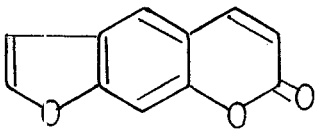
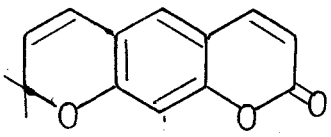
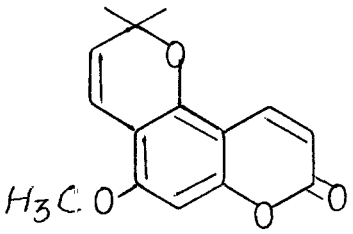


VI



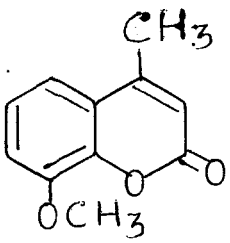
VII

in formation of the third ring, for example alloxanthoxyletin (VIII).

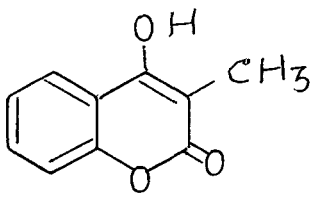


VIII

Many coumarins carry isoprenoid side chains of one, two or three units, either on one of the free positions on the benzene ring or in an ether linkage. O-Methylation in coumarins is of common occurrance but C-methyl derivatives are rare. 4-Methyl-8-methoxy coumarin (IX) and 3-methyl-4-hydroxy coumarin (IXa) are typical examples of C-methylated coumarins.



IX



IXa

Methods of Isolation:

Coumarins are usually isolated from the natural source by extraction in a soxhlet with petroleum-ether or benzene. Alcohol is avoided as it extracts too much of phenolic products and makes purification very difficult. They usually crystallise from the petroleum ether extract either directly or on concentration. Failing this they have to be freed from non-acidic material by extraction with dilute alkali, though here there is a danger of isomerisation if a hydroxyl group at 5-position is present. Fractional crystallisation serves in some cases in separation of the coumarin mixture present in the extract though usually this is a very tedious procedure, and to avoid large number of crystallisations chromatographic methods of separation are used. Here it is convenient to resolve the mixture first on chromatostrips which gives an indication of the number of coumarins present and the adsorbent to be used for large scale separation on columns. W.L.Stanley[4], who employed silicic acid for the separation of citrus oil coumarins, has pointed out that the same solvent mixture can not always be used in separation on columns as on chromatostrips. According to him for a coumarin which gives an R_f value of 0.6 on chromatostrips with a particular solvent mixture, the eluate to be used on column should be so adjusted that it would have given an R_f value of 0.2

on plates.[5] Usually petroleum ether-benzene, petroleum ether-ethyl acetate and ethyl acetate-acetone serve as good eluants. Repeated chromatography is often necessary if the mixture contains too many coumarins or if the coumarins have about the same R_f value.

Ultraviolet spectra of coumarins are fairly characteristic and serve to distinguish them from other impurities. Though there is no specific reagent for coumarins, they usually give fluorescent deep yellow solution with alkali.[6] Phenolic coumarins do not always give a colour reaction with ferric chloride.

The double bond of the side chain can easily be reduced over palladium-charcoal, the 3,4-double bond requires hydrogenation under pressure. The dihydrocoumarins, in turn, can be dehydrogenated smoothly to the corresponding coumarins by heating at 230° with palladium-charcoal,[7] 4',5'-dihydrofurocoumarins can also be similarly dehydrogenated.

Selective ozonolysis is also possible as the side chain undergoes cleavage more readily than the furan ring and the latter more readily than the 3,4-double bond. Though ozonolysis is generally employed to obtain information on the position of the double bond in the side chain, chromic acid in acetic acid is also used for

the same purpose and cleaves the terminal isopropylidine unit as well as the corresponding hydroxy, dihydroxy and epoxy derivatives. Alkaline hydrogen peroxide destroys the benzene ring to give furan-2,3-dicarboxylic acid, but as the presence of furan system can be usually established in other ways (red colouration on heating in concentrated hydrochloric acid with p-dimethylamino benzaldehyde, ultraviolet and infrared spectra) and gives no information on the position of the furan ring it is now seldom used.

Ultraviolet Spectra:

Spectroscopic data on a large number of naturally occurring coumarins has been recorded in literature[8,9,10] and attempts have also been made by several authors to establish a correlation between absorption pattern and structure[11-14], which could be helpful in gaining some insight into the structure of unknown compounds of this group. However, this has not been entirely successful and structural assignments on this basis have to be made with some caution. The spectra of coumarins are, however, sufficiently characteristic to allow members of this group to be distinguished from other phenolic plant products, taken together with the absence of diagonistic colour reactions.

Simple substituted coumarins as well as the parent compound show two zones of absorption of about equal intensity, the first in the 270-290 mμ(log ϵ 4.0) the second in the 300-330 mμ(log ϵ 3.5) regions. The spectra are essentially similar to those of the corresponding trans cinnamic acids as can be seen from the following values:

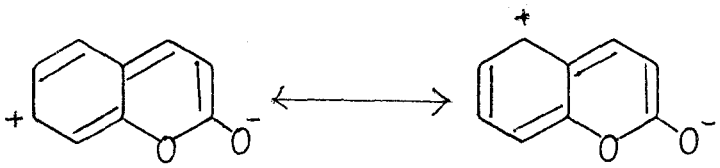
Name	λ max(mμ)	Log ϵ
2-Hydroxy-6-methoxy-trans-cinnamic acid	225,300	4.15, 4.3
2,5-Dimethoxy-trans-cinnamic acid	220,278,340	4.12,4.17,3.85
2,4-Dihydroxy-trans-cinnamic acid	216,290,330	4.08,4.0,4.05
2,3-Dihydroxy-trans-cinnamic acid	218,286,320	4.17,4.3,3.65

Introduction of hydroxyl or methoxyl in 5,6 or 8-position of the coumarin molecule does not bring about the expected increase in intensity and the long wave-length shift. In 7-hydroxycoumarin (I), however, the two maxima seem to coalesce to give a single high intensity maximum, at 325 mμ.[12]

This is in contrast to the benz-γ-pyrone system such as that of the flavonoids where substitution specially in para position to the carbonyl is associated with a marked bathochromic shift and an increase in

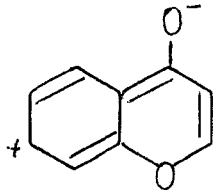
Name	λ max.(mμ)	log ε
Coumarin	275,312	4.1,3.5
5-Hydroxy coumarin	245,300	3.81,4.07
5-Methoxy coumarin	245,301	3.76,4.1
6-Hydroxy coumarin	224,276,348	4.3,4.02,3.6
6-Methoxy coumarin	225,275,335	4.4,4.04,3.6
7-Hydroxy coumarin	325	4.0
7-Methoxy coumarin	218,318-23	4.11,4.17
8-Hydroxy coumarin	210,254,289	4.05,3.94,4.
8-Methoxy coumarin	251,286	3.9,4.1

intensity.[15] This may be interpreted to mean that the contribution of polar structures such as X and XI is



X XI

not as pronounced here as in the case of benz-γ-pyrones (XII).



XII

This behaviour can be compared with that of acetophenone and benzoic acid.[16] The K and B bands of the former occur at 240 mμ(log ε 4.11) and 278 mμ(log ε 3.04)

respectively, whereas of the latter are at 230 mμ ($\log \varepsilon$ 4.0) and 270 mμ ($\log \varepsilon$ 2.90) respectively. This is in agreement with the fact that delocalisation of electrons from acidic functions is less favoured than that from the keto functions.

According to Manginin and Passerini[10] the absorption maximum in the region 300-333 mμ is due to the combination of all the resonating structures of coumarin and the maximum in the region 270-290 mμ is due to antisymmetric combination of the polar structures only.

Among disubstituted coumarins Böhme et al[14] observed a bathochromic shift for 6,7-, and a hypsochromic shift for 5,8-dihydroxy coumarins. Methylation of hydroxyl, in agreement with previous findings in phenolic compounds, does not alter the spectrum appreciably. Acetylation also follows the general rule and reverts the spectral pattern to that of the parent compound.

Studies in alkaline medium showed that coumarins are not stable in aqueous alkaline medium, undergoing ring opening along with oxidative changes to a quinoidal system, specially when resorcinol or hydroquinone is formed on ring opening. However, according to Böhme the lactone ring of coumarins and substituted derivatives is stable towards alcoholic sodium methoxide. They further observed that ring opening under these conditions begins

to be noticeable after about 24 hours and proceeds much more slowly in the case of 5 and 7-hydroxy coumarins than in the 6,8-substituted derivatives which they associate with the enhanced stability of the polar structures.

This makes it possible to infer the presence of free phenolic groups and can give an indication of their position as well. (Ferric chloride colour is generally not very clear with coumarins).

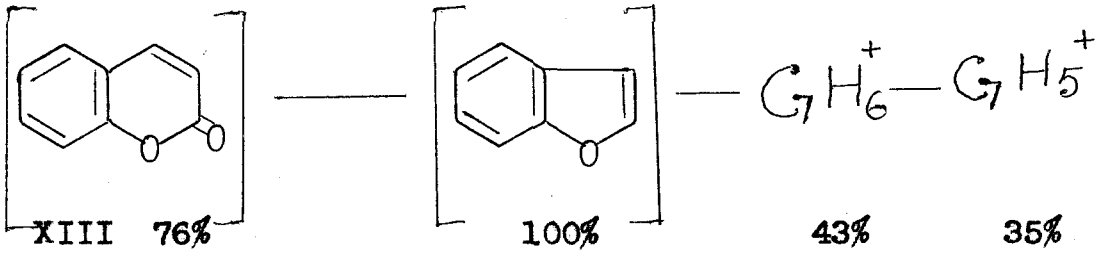
Furocoumarins have an additional band which occurs at the same value 247 mμ in both psoralen and angelicin. However, psoralen has a further low intensity maximum at 330 mμ(log ε 3.4). The 330 mμ band is absent in the spectrum of xanthotoxol. The presence of 247 mμ band is suggestive of the presence of furan ring but the maximum persists even on hydrogenation of the furan ring.

Mass Spectroscopy:

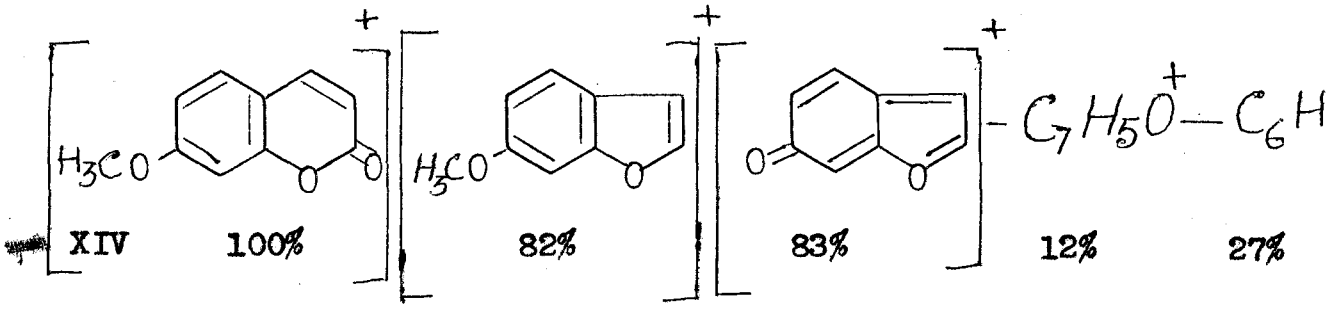
Mass spectrometry has now found extensive use in the study of plant products but not much work has been done in the field of oxygen heterocyclic compounds. Barenes and Occolowitz[17,18] have studied mass spectra of a number of simple compounds of this class. According to them coumarins under the impact of electrons readily lose CO from the pyrone ring to form an ion having benzofuran structure. This has been established by the very close

similarity in spectra of the two compounds. Their findings were later confirmed by Vulfson et al.[19]

In the spectrum of coumarin (XIII) there is a strong molecular ion peak (146, 76%) and a base peak 28 units lower (118,100%) formed directly from it by the loss of oxygen as CO.

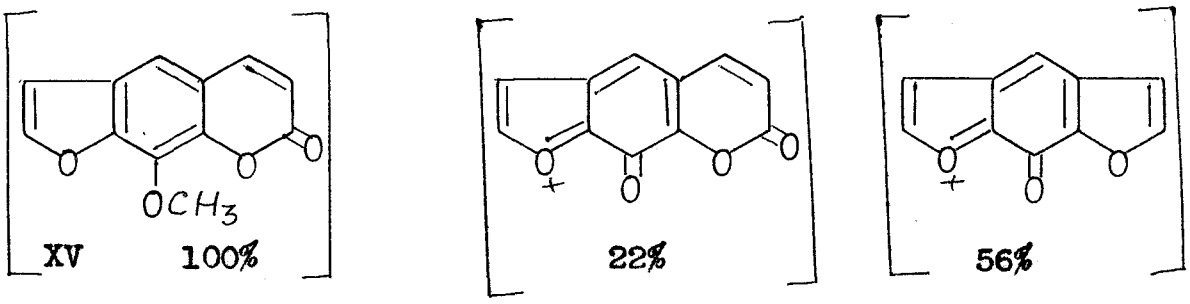


The second oxygen atom is again lost as CO as shown by the metastable transition 118-90=28 to give a strong peak at m/e 90 (43%) corresponding to C_7H_6 ion of uncertain structure. There is another strong peak at m/e 89 (35%) due to loss of a hydrogen atom from the ion of mass 90. The spectrum of dihydrocoumarin bears some resemblance to that of coumarin, both showing two consecutive losses of 28 units, probably due to the removal of oxygen atoms as CO, but the presence of two methylene groups in dihydrocoumarin gives rise to the possibility that one of the 28 units may arise from removal of an ethylene molecule. The fragmentation in the case of simple substituted derivatives follows the expected pattern. The spectrum of 7-methoxycoumarin (XIV) gives the molecular ion as base peak (176, 100%) and then a peak at m/e 148(82%)



due to loss of oxygen as CO. The ion thus formed instead of splitting out oxygen as CO loses a methyl radical to give a strong peak at 133 (83%) due to an ion probably stabilised by a quinonoid structure. The remaining two oxygen atoms in the ion m/e 133 are lost, as expected, as CO to give finally the phenyl radical ion.

Fragmentation of furocoumarins follows the same scheme. The spectrum of angelicin has the molecular ion peak at m/e 186 (73%) and a peak 28 units lower (158, 100%), due to loss of CO, as the base peak. The remaining oxygen atoms are then removed as CO. Wherever in furocoumarins the loss of a methyl radical from a methoxyl gives rise to a quinonoid structure there is no fission between the aromatic ring and ether oxygen. For example xanthotoxin (XV) in its spectrum shows molecular ion as the base peak (216, 100%) and the ion due to the loss of CH_3 (201).



SYNTHESES OF FUROCOUMARINS

The reported syntheses of furocoumarins, with few exceptions, have followed two general routes.

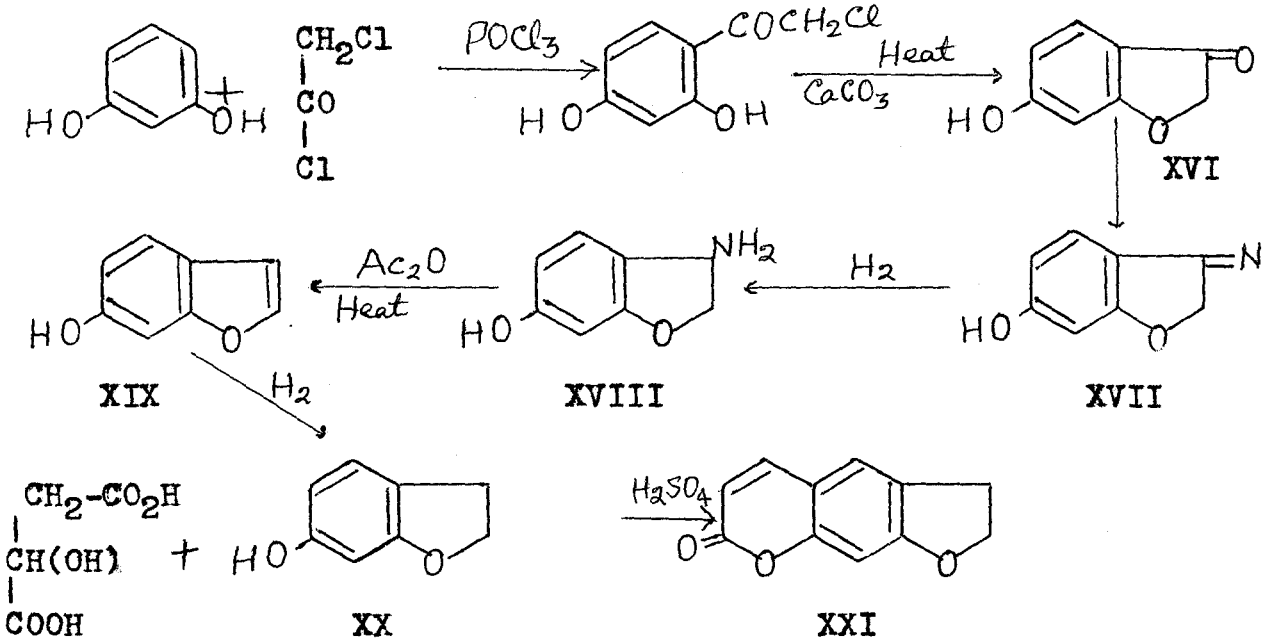
(i) The starting material contains the furan or dihydrofuran ring and the lactone (α-pyrone ring) is subsequently introduced.

(ii) The starting material contains the lactone ring (benz-α-pyrone) and the linear or angular furan ring is then introduced.

Both these methods were initially developed by Spath and subsequently modified by others.

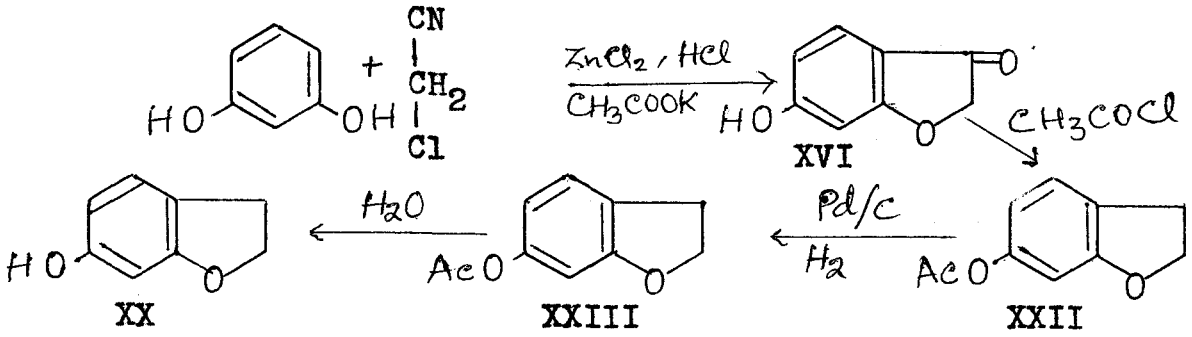
I: From 6-hydroxycoumarans:

This method was first described by Späth[20] in 1936 for the preparation of psoralen (VI) and its derivatives using Pechman's[21] coumarin synthesis. The starting material, a suitably substituted benzofuran was itself prepared by him through the following sequence:

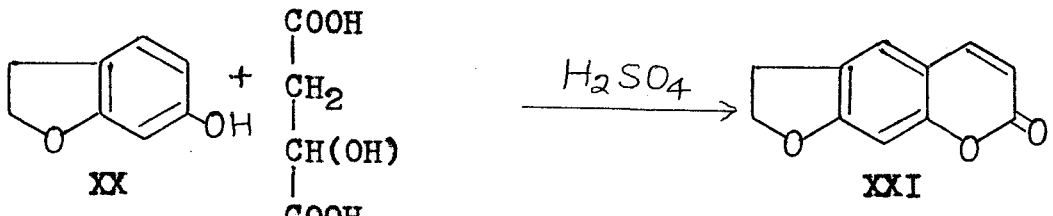


Conversion of the coumaranone (XVI) to coumaran (XX) was brought about by Sonn's method[22] and involved the elimination of NH_3 from the amino compound (XVIII) by heating the compound or its aqueous solution for 2 hours.

This general procedure was modified to some extent by Horning and Reisner[23] who used the Hoesch[24] reaction with chloroacetonitrile for the preparation of ketone XVI and then reduced this catalytically. They found that the catalytic hydrogenation proceeded with good yield only when compound XVI had previously been acetylated. Condensation



of XX with malic acid in presence of concentrated sulphuric acid then gave 2',3'-dihydropsoralen (XXI), although the yield of the pure material was low.

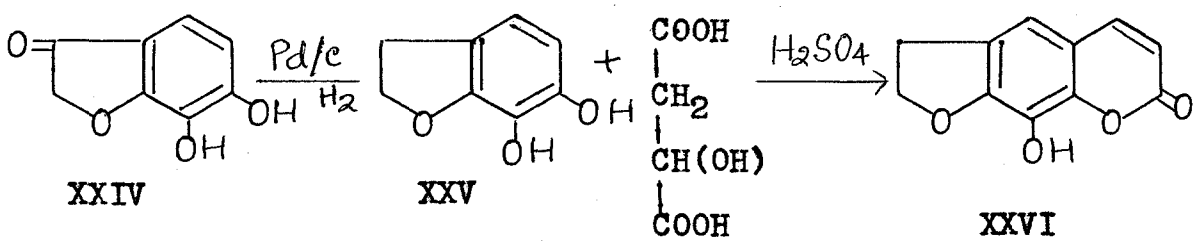


Horning and Reisner also investigated the dehydrogenation of 2',3'-dihydropsoralens.[25] Catalytic

dehydrogenation of heterocyclic compounds containing nitrogen as the hetero atom had been extensively studied but very little information was till then available on the dehydrogenation of oxygen heterocycles. The dehydrogenation of dihydrocoumarins and a few hydrogenated furocoumarins was first investigated by Spath.[7] The method generally consisted in heating a mixture of the compound with palladium black to 170° or higher, followed by distillation to remove the product from the catalyst, however, yields were low and usually below 20%.

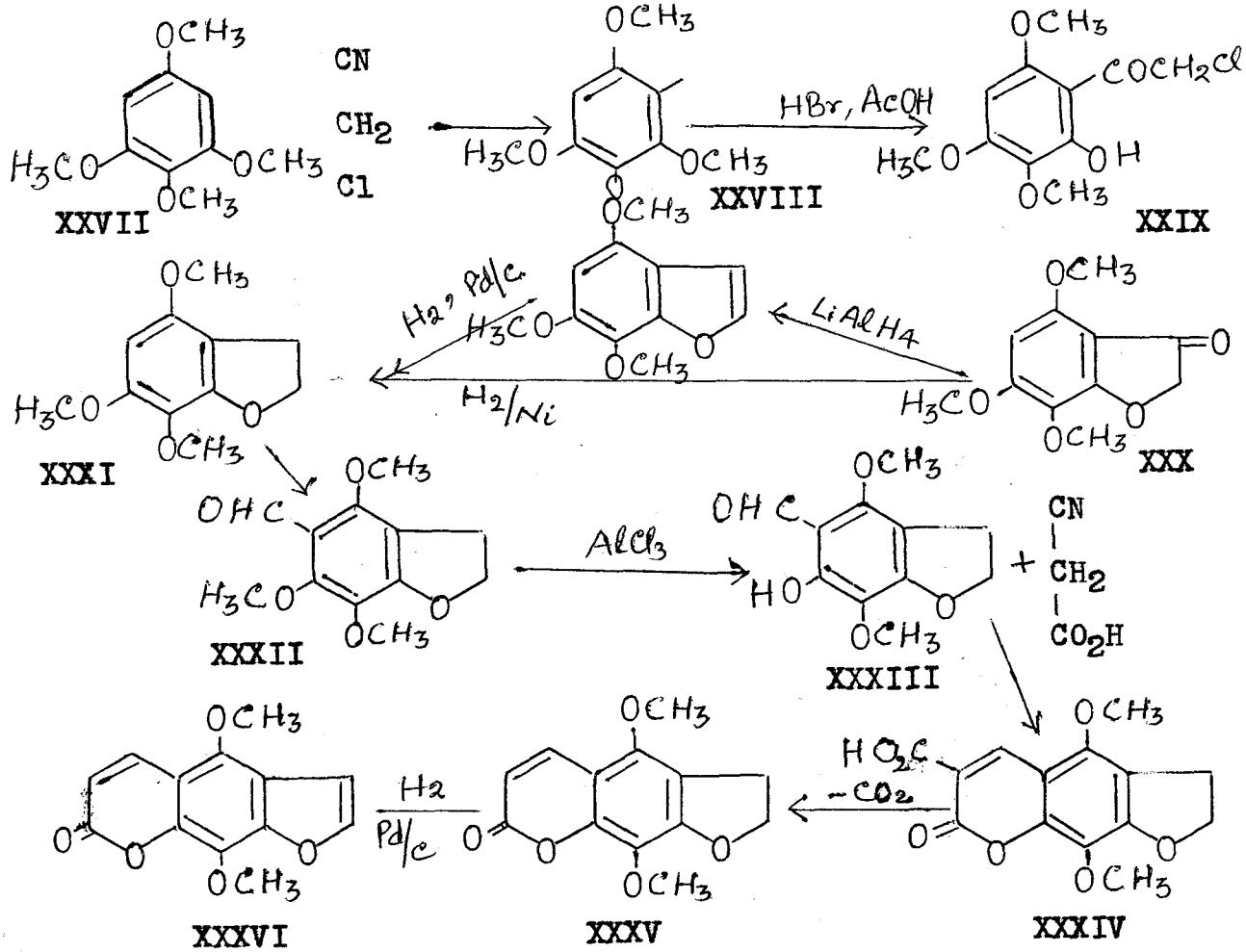
According to Horning dehydrogenation can be carried out with better results by the use of palladium black in some high boiling solvent and he observed that best yields were obtained with diphenyl-ether as solvent.

Lagercrantz[26] synthesised dihydroxanthotoxol (XXVI) from 6,7-dihydroxycoumaran-3-one(XXIV) by the same procedure. He, however, found that acetylation of the hydroxyl prior to hydrogenation was not essential. 6,7-Dihydroxycoumaran (XXV) was then converted to dihydroxanthotoxol (XXVI) by Pechmann synthesis.

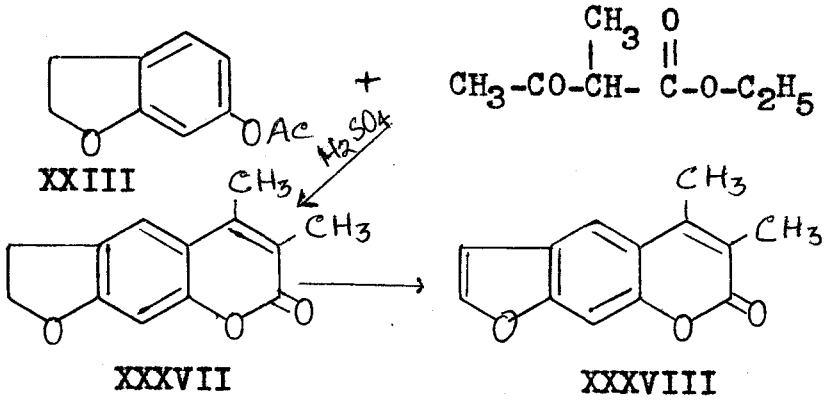


XXIV XXV XXVI

Yields in the Pechmann reaction are mostly low and cyano acetic acid and ethyl acetoacetate have been used with improved results. Starting from 1,2,4,6-tetramethoxy benzene (XXVII) Horton and Paul[27] synthesised isopinpinallin (XXXVI) and its derivatives through the following steps (XXVII-XXXVI). The coumaran-3-one (XXX) could be converted to the coumaran (XXXI) either by (i) direct hydrogenation of the compound or its acetate in presence of Ni, or (ii) by reduction with lithium aluminium hydride followed by hydrogenation over palladium charcoal.



Esse and Christensen[28] have described the synthesis of a series of 3,4-dialkyl-2',3'-dihydropsoralenes and have also dehydrogenated these to the corresponding derivatives of psoralen. 6-Acetoxycoumaran (XXIII) was condensed with an α-alkyl-β-keto ester to from 3-alkyl-2',3'-dihydro-4-methyl psoralen. With the exception of 2',3'-dihydro-4-methyl-3-myristyl psoralen all other compounds prepared were dehydrogenated by refluxing in phenylether in the presence of palladium charcoal.

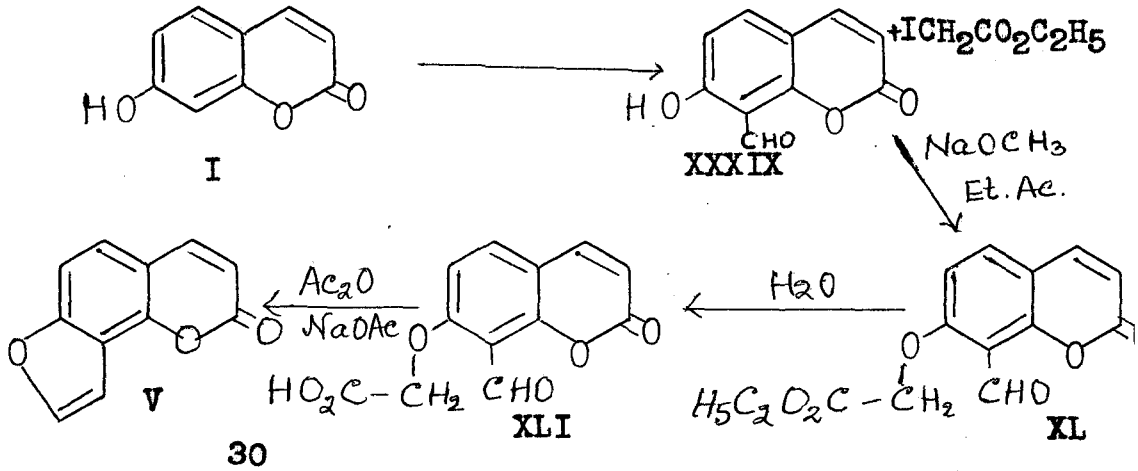


XXIII XXXVII XXXVIII

II: from 7-hydroxy-o-formylcoumarins:

Starting from 7-hydroxy-8-formyl coumarin (XXXIX) Spath[29] in 1935 synthesised angelicin (V). XXXIX was obtained by the action of hexamethylene tetramine on umbelliferone (I) in glacial acetic acid for 5 hours. Condensation of this with iodoethylacetate and subsequent hydrolysis of the carbethoxy methoxy derivative gave the corresponding carboxy methoxy formyl derivative (XLI) which

was cyclised with acetic anhydride sodium acetate to give angelicin (V).



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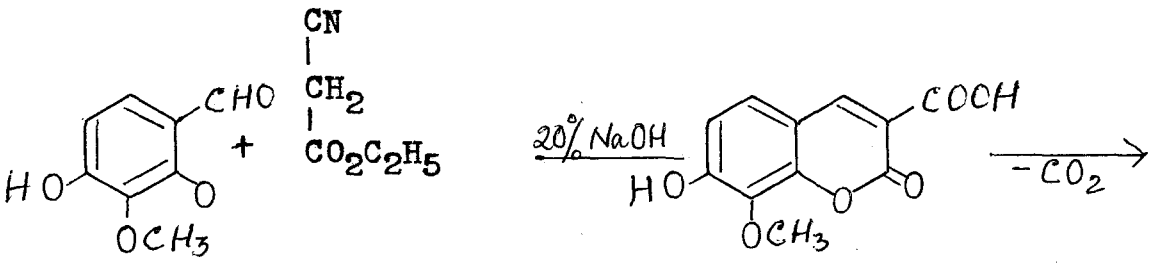
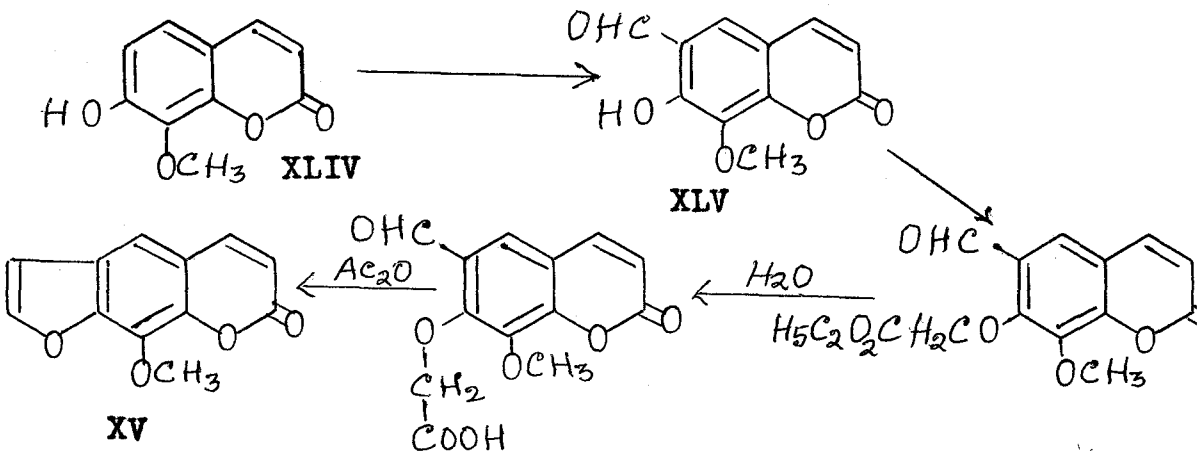
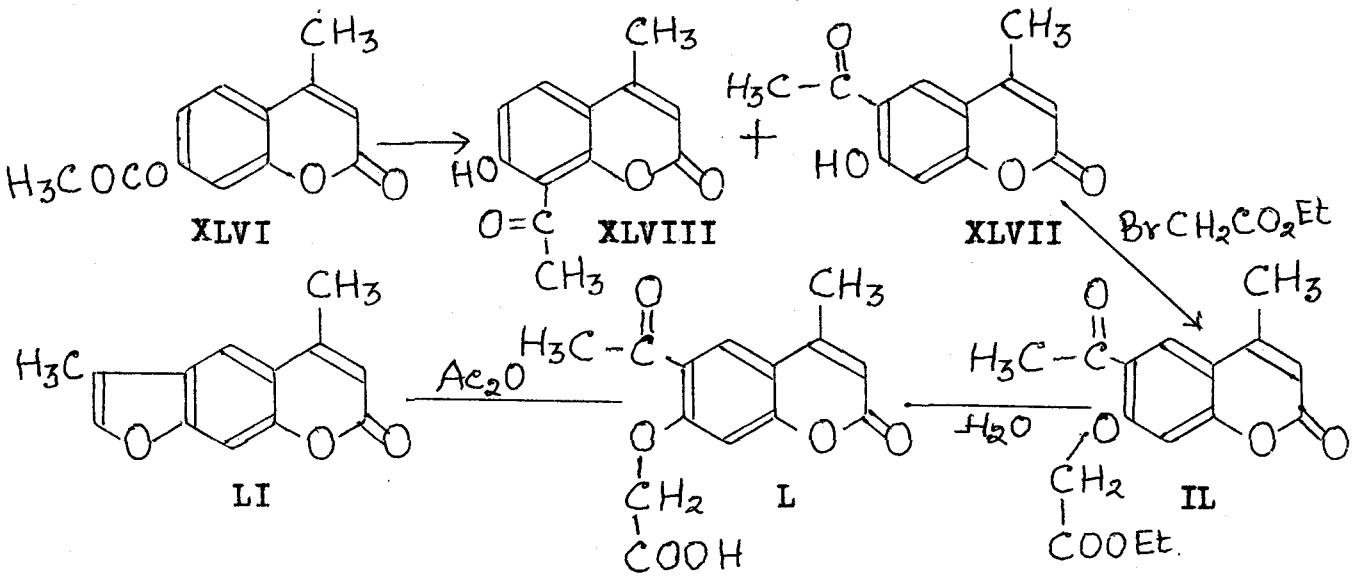
Rodighiero in 1956 synthesised xanthotoxin (XV) by the same procedure. 2,6-Dihydroxy-1-methoxy-5-formylbenzene (XLII) was condensed in alkaline medium with cyanoethyl-acetate to give 7-hydroxy-8-methoxy-3-carboxycoumarin(XLIII) which was decarboxylated by heating at 270°/100 mm with calcium carbonate to yield 7-hydroxy-8-methoxy coumarin (XLIV). On treatment with hexamine in acetic acid (XLIV) was converted to its o-formyl derivative (XLV), condensation of which with bromoethylacetate and subsequent cyclisation of the product with acetic anhydride gave xanthotoxin.

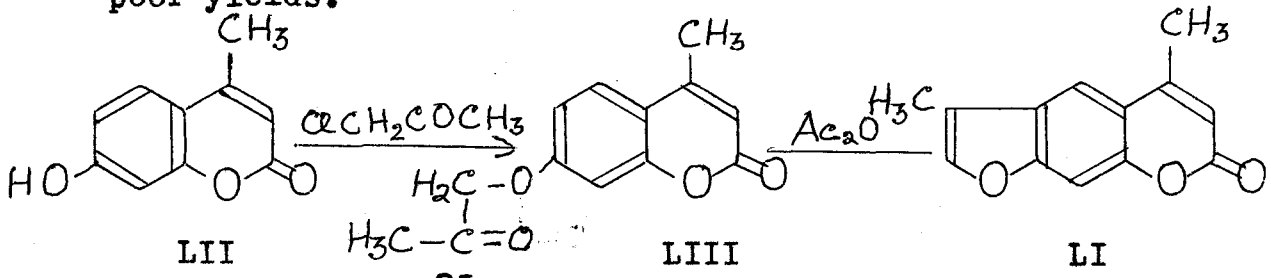




Limaye[31] prepared angular furocoumarin derivatives from 7-hydroxy-4-methyl-8-acylcoumarin by its condensation with chloroacetic acid followed by ring closure with acetic anhydride and decarboxylation to the angular furocoumarin.[32] The linear furocoumarins synthesised by him involved 6-acyl-4-methyl umbelliferone (XLVII) as intermediate, which was readily obtained by Fries rearrangement of 4-methyl umbelliferone acetate (XLVI). The 8-isomer formed alongside in the same reaction was separated by fractional crystallisation. On condensation with bromoethylacetate in sodium ethoxide, XLVII gave the carbethoxy methoxy derivative (IL) of the acylcoumarin. This was hydrolysed to the corresponding carboxy methoxy acyl coumarin (L) which could be smoothly cyclised with acetic anhydride to 4,3'-dimethylpsoralen (LI). This procedure was utilised for the synthesis of a number of furocoumarins.[33]



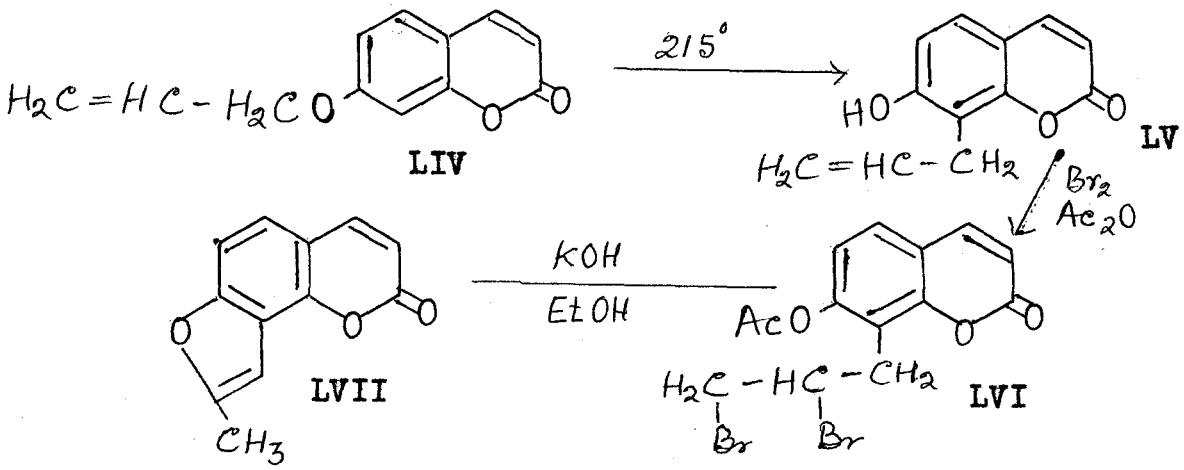
A useful variation in this method was introduced by Ray et al[34] who substituted chloroacetone for bromoethyl-acetate and thus avoiding Fries rearrangement which gives poor yields.



Shah and Shah[35] prepared psoralen derivatives without any substitution in the pyrone ring, using 7-hydroxy-8-acetyl or benzoyl coumarin as an intermediate, condensation of which with bromoacetic ester and subsequent cyclisation gave 3'-methyl or phenyl isopsoralen.

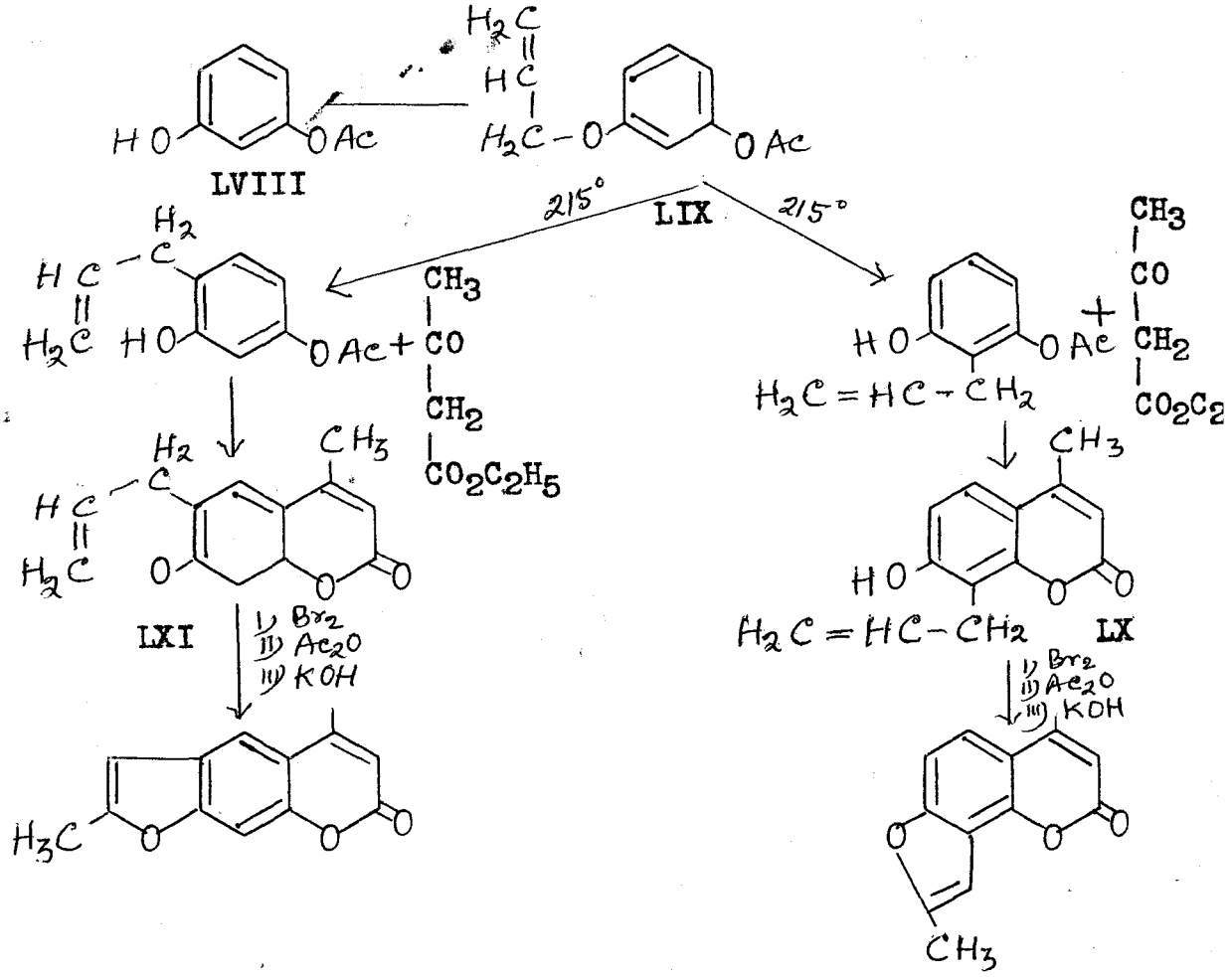
Recently Kaufman[36,37] has described a new method for the synthesis of linear and angular furocoumarins, involving o-allyl-7-hydroxy-coumarins (LV) as intermediates. This method is based on the known conversion of o-(β,γ -dibromopropy

phenylacetate to 2-methyl benzofuran.[38] The 8-allyl-7-hydroxy coumarin (LV) can be easily obtained by Claisen rearrangement of 7-allyloxy coumarin (LIV) at 215°.[39] Bromination and acetylation of LV gave o-(β,γ-dibromopropyl)-7-acetoxy-coumarin (LVI) which on cyclisation with potassium hydroxide in ethyl alcohol afforded 2'-methyl-angelicin (LVII).

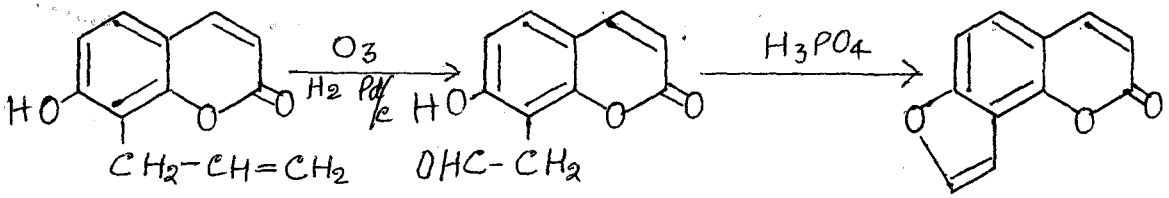


As migration of the allyl group takes place preferentially to the 8-position this method can be applied for the synthesis of linear furocoumarins only when the 8-position has been blocked. This is partly avoided by effecting the migration before the α-pyrone ring cyclisation and separating the two isomers which are now obtained in about equal amounts. Thus resorcinol menoacetate (LVIII) was converted to 3-allyloxyphenyl acetate (LIX) which underwent Claisen rearrangement at 215°. The hydrolysed products were condensed with ethylacetoacetate and the mixture of

8-allyl-7-hydroxy coumarin (LX) and its 6-isomer(LXI) were resolved by column chromatography on alumina and then converted as above to angular and linear furocoumarins by the above sequence of reactions.

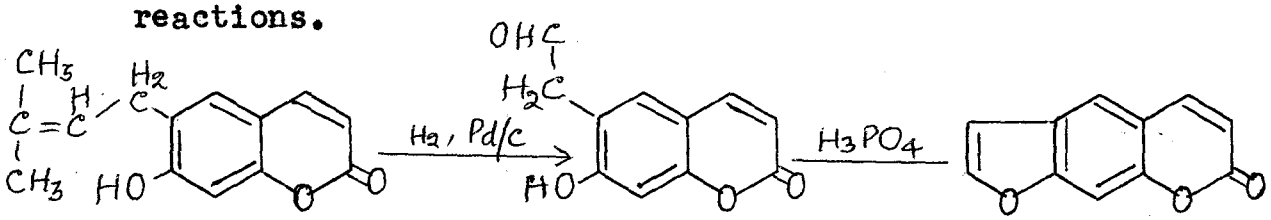


8-Allyl-7-hydroxycoumarin (LV) was oxidised by ozonised oxygen in ethylacetate and the ozonide reduced over palladium-carbon to give 7-hydroxycoumarin-8-acetaldehyde (LXII) which was cyclised to angelicin [40] by polyphosphoric acid at 100°.



LV LXII V

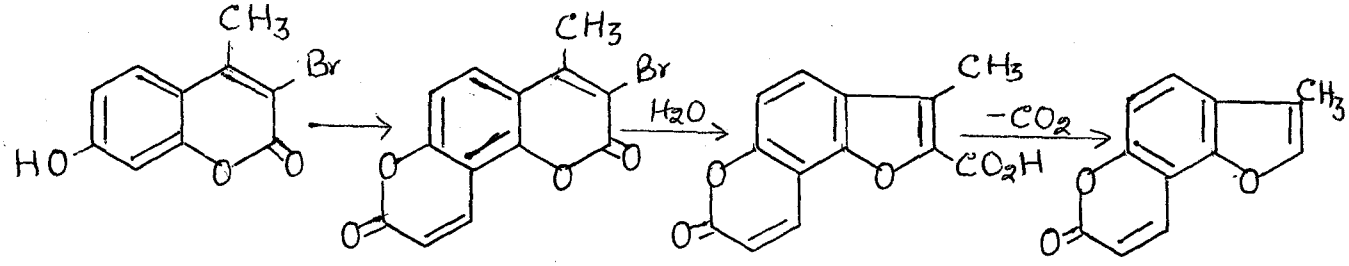
Similarly psoralen was obtained when demethyl subrosin (LXIII) was subjected to the above sequence of reactions.



LXIII VI

III: From Pyrono-coumarins:

Trivedi and Sethna[41] have used coumarino-7,8-α-pyrones as intermediates in the preparation of furocoumarins. 7-Hydroxy-3-bromo-4-methyl coumarin (LXIV) was condensed with malic acid to afford 3-bromo-4-methyl coumarino-7,8-α-pyrone (LXV). Hydrolysis with ethanolic potassium hydroxide gave the furocoumarin carboxylic acid derivatives which on decarboxylation gave the furocoumarin (LXVII).



LXIV LXV LXVI LXVII

PHYSIOLOGICAL PROPERTIES

Coumarin is narcotic for some animals and a sedative and hypnotic for mice.[42] Some larger animals can be killed by coumarin but moderate quantities have no very marked effect.[43] Spath found that certain coumarins have a slight toxic effect on rats, mice and guinea-pigs. Spath and Kuffner[44] demonstrated that natural coumarins have a powerful effect on fresh-water fish. Simpler coumarins have little action but the complex members, e.g. furocoumarins are toxic in very small doses.

Apart from this the more recent pharmacological investigations have been directed mainly towards the following:

(a) Skin photosensitizing activity.

(b) Anticoagulant activity.

(c) Antibacterial activity.

Skin Photosensitizing activity:

Skin photosensitizing action of certain plant juices and extracts has a very early history but the correlation of this property to the presence of furocoumarins in these plants was first definitely established in 1938 by Kuske.[45] In 1947, Fahmy and Abu-Shady[46,47] isolated xanthotoxin (XV), bergapten and imperatorin from Ammi majus seeds, a drug long in use in Egypt in the treatment of leucodermic spots. Subsequently El Mofty[48] carried out extensive clinical trials and found it effective in the treatment of Vitiligo.

The structure-activity studies carried out by Musajo and Rodighiero[49,50] and by Pathak[51,52] showed that the maximum activity is possessed by the parent compound, psoralen (VI) whereas the other structurally related compounds have reduced activity. Comparative studies have shown that the presence of free phenolic groups deactivates the molecule but alkylation offsets this to some extent. The activity is also gradually reduced with the lengthening of the alkyl side chain. Again introduction of nitro-, amino, acetyl amino groups also deactivates the parent compound. Nuclear substitution with methyl groups at 4, 2', 3' or 8-position may or may not inhibit activity but a methyl group at 3-position invariably brings this about. They have also shown that linear furocoumarin structure is more active than the angular one and that psoralen (VI) is much more active than angelicin (V), isobergapten and allo-bergapten.

Little is known of the mechanism of the photodynamic action of these compounds.[53] Fowlks believes that light is absorbed by the photosensitizer molecule or a complex of the photosensitizer with protein or nucleic acid. This may be followed by:

(i) Chemical combination of Photosensitizer with a sensitive cell constituent. Or

(ii) Oxidation of the sensitive cell constituent. Or

(iii) Indirect excitation of the cell constituents which through chemical changes inhibits vital cellular activities.

Pathak and Fellman[54] noted that furocoumarins having photosensitizing action possessed absorption and fluorescence peaks of 320-360 and 420-460 mμ respectively and those absorbing outside this range were inactive. They have also shown that long wave length ultra violet radiation is required to initiate the response of the skin to the coumarins It is believed that the skin response is associated with the capture of the radiant energy of proper wave length and that any change in the molecular structure, substitution pattern etc. which disturbs this specificity leads invariably to decreased activity. Thus absorption of light of a specific wave length followed by emission of light of another wave length in intimate contact with sensitive cellular component is thought to be crucial to the successful photosensitizing action. Radiogheiro and Capellina[55] have shown that furocoumarins are dimerized under the influence of ultra violet light, but the dimers are inactive. Pathak et al[56] have suggested that free radicals may be generated from the excited furocoumarins molecule under ultra violet light.

An interesting chemical reaction of flavin mononucleotide (FMN) with furocoumarins was observed by Musajo et al[57] in 1961. They found that FMN reacts only with photodynamically active furocoumarins and the reaction

products appear to have undergone some modifications in the furan ring. Thus FMN was found to possess antagonistic action toward the erythemel response expected from psoralen type molecule.

In spite of all these investigations the clear cut mechanism whereby furocoumarins function in the treatment of leucoderma is unknown. Although the biological reactions leading to the formation of the skin pigment, melanin[58,59] have been clarified, there is no indication yet that coumarins are involved here.

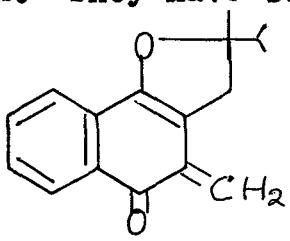
Anticoagulant Activity:

While studying the toxic principle of spoiled cloverhay, Link[60] in 1941 showed that coumarin derivatives possess anticoagulant properties. This toxic principle, causing serious hemorrhagic conditions on cattle was found to be dicumarol, 3,3'-methylenebis-(4-hydroxy coumarin) (II). Following this observation the relationship between structure and the anticoagulant activity was examined by several workers.

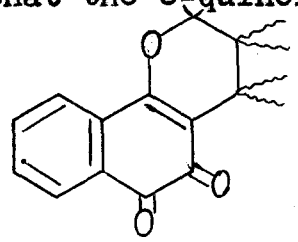
Link[61] found that the minimum structural requirement for the anticoagulant activity was the presence of intact 4-hydroxy coumarin nucleus with a substituent at 3-position. Later on Mentzer and his associates[62] also observed that this type of arrangement was needed, although subsequent work has shown it to be a non-essential feature.

The anticoagulant activity of the coumarin molecule is considered to be antagonistic to vitamin K activity in which the anticoagulant competes with vitamin K in the blood clotting mechanism. In vivo it has been shown that presence of coumarin reduces the blood prothrombin concentration thereby increasing the blood clotting time. Since coumarins are structurally similar to vitamin K they can compete with vitamin K for the apoenzyme and thus exert antivitamin K activity.

In keeping with the above view Chielewska and Cieslak[63] have suggested that the structure essential for vitamin K activity is expressed by formulae LXVIII and LXIX. LXVIII arises by the biological oxidation of the side chain of vitamin K. They have suggested that the o-quinoidal group,



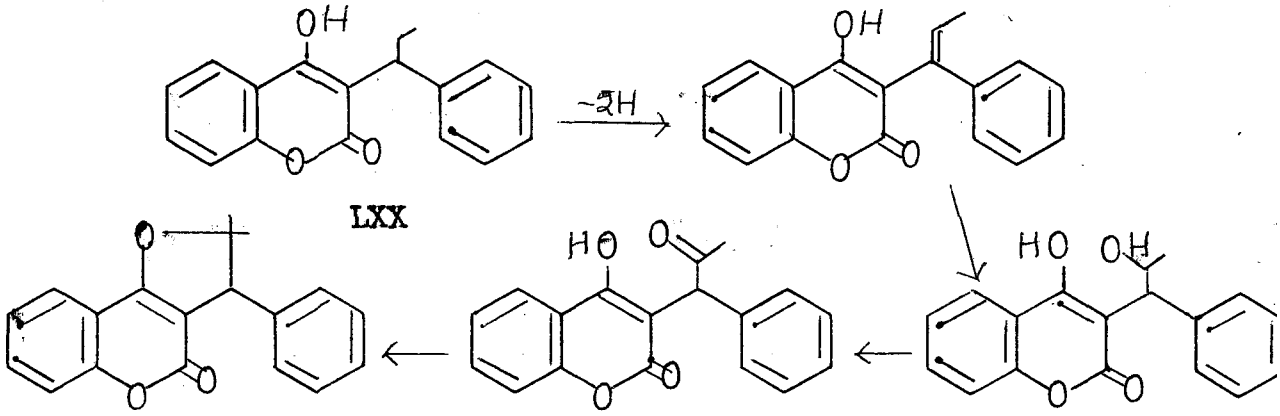
LXVIII



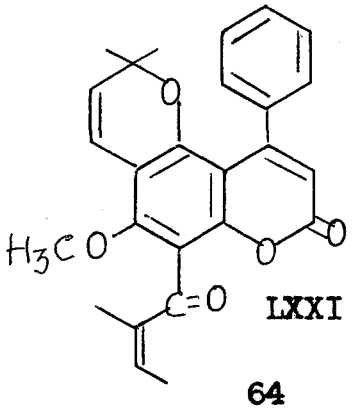
LXIX

$O{=}C{-}C{=}CH_2$, acts as the active centre, the hemiketal group serves for the attachment to the protein part of the enzyme. According to this view the coumarin type antivitamin K compounds possess only the hemiketal linkage and no active centre, and can thus successfully compete with vitamin K for the apoenzyme. But the two active coumarins, dicumarol (II) and 3-(1'-phenyl-n-propyl)-4-hydroxy coumarin (LXX) do not possess the hemiketal linkage, therefore they do not fit

into this hypothesis. It has been suggested that these probably first undergo biological oxidation in 2-position,



but even so there are certain antivitamin K compounds with an anticoagulant activity superior to that of dicumarol (II) whose activity can not be explained by their hypothesis. Calophylloide (LXXI), the naturally occurring coumarin is an excellent anticoagulant but is not a 4-hydroxycoumarin.



LXXI

64

Recently Arora and Mathur in their studies of a number of coumarin derivatives have generally supported the Chielewsk hypothesis in the case of 4-hydroxycoumarins. They have found that methylation of 4-hydroxy group potentiates the activity but additional loading of the molecule can inhibit

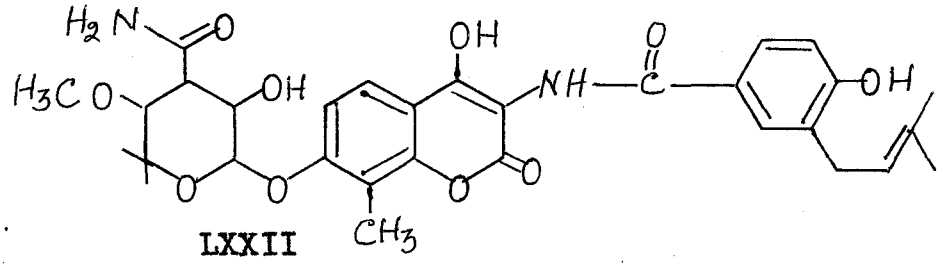
the af

the activity depending upon the location of the groups. Methylation at 8-position had a special potentiating effect. According to them the general features that could be essential for the antivitamin K activity are molecular shape, six membered heterocyclic system such as a cyclic acetal, a substituent at the 8-position and etherefication of free phenolic groups. They, however, believe that the activity is governed not only by individual structural features but by a combination of several features which can not be explained as yet.

Antibacterial Activity:

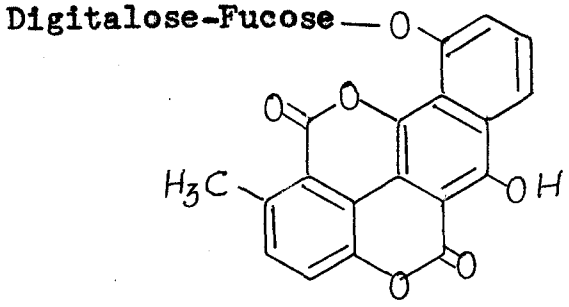
Antibacterial activity is also shown by some coumarins. Coumarin itself is a mild antibacterial substance but dicumarol (II) has excellent activity against certain bacteria.[65,66] Cavallito[67] suggested that the predominantly Gram positive activity of lipophyllic dicumoral (II) is accounted for by the association of hydrophylic properties with Gram-negative activity.and lipophylic properties with Gram-positive activity.[68] Ukita and co-workers observed excellent activity by 3-acetyl-4-hydroxy coumarin against certain bacteria.

The most important antibacterial agent of this type is novobiocin (LXXII)[69] isolated from fungae metabolite of streptomyces niveus and is widely used in medicine. Amide derivatives of novobiocin and 3-amino-4,7-dihydroxy-8-methyl coumarin are commonly used in oily oihtments due to their better oil solubility.



LXXII

Chartreusin (LXXIII) is another coumarin antibiotic isolated from the metabolite of streptomyces chartreusis.[70] It is also predominantly effective against Gram-positive organism but it is toxic and has not been commercially exploited.[30]



LXXIII

D I S C U S S I O N

DISCUSSION

Heracleum candicans:

Heracleum candicans grows abundantly at higher altitudes in Kashmir[71] where it is used in folk medicine for a variety of skin affections. This plant was collected in October and the air dried roots were extracted soon after collection to avoid changes in the contents following prolonged exposure to air and light. The extracts were preserved in dark as the TLC pattern was found to vary after prolonged exposure to light.

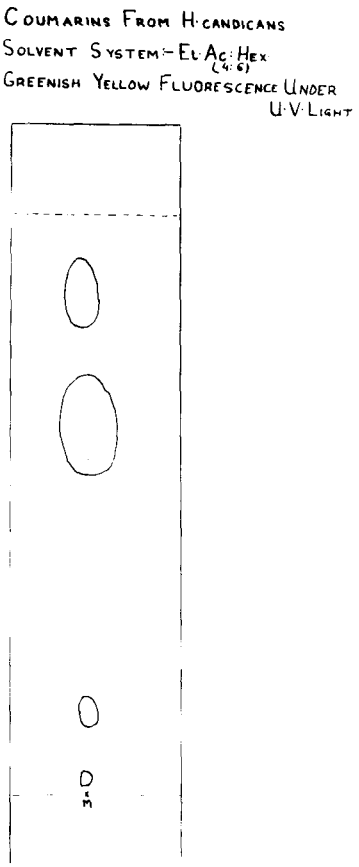


Fig. I

The dried roots were extracted first with petroleum ether and then with benzene. The petroleum ether extract deposited a solid on slight concentration and cooling whereas evaporation of the benzene extract left a gummy residue which was purified by column chromatography over silica gel and crystallised from ethyl acetate-hexane mixture.

Thin layer chromatography[72] (Fig. 1) of the solid obtained from the petroleum ether extract showed four fluorescent spots under an ultraviolet lamp. Resolution of the mixture over silica gel strips was sufficiently sharp to suggest that it would be suitable adsorbent for separation of larger amounts on columns but it was later found that acetic acid deactivated alumina gave somewhat better results.

On elution of the column with petroleum ether and petroleum ether-benzene mixtures the following products were obtained from different fractions:

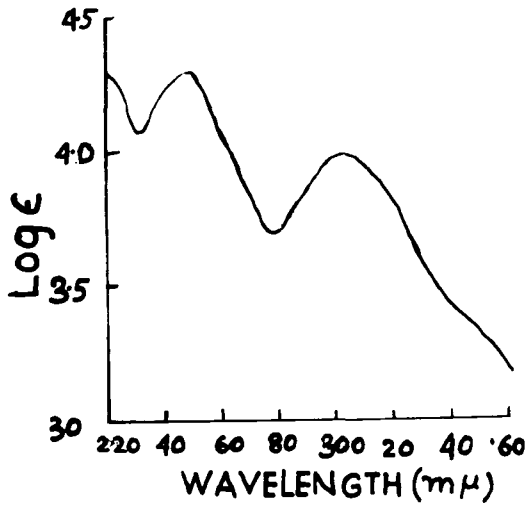
Petroleum ether 100%	Solid	melting point 53-54°
Petroleum ether-benzene (5:1)	Rhombic crystals.	melting point 82-83°
Petroleum ether-benzene (1:1)	Crystals from methanol.	melting point 111°

The solid obtained from the benzene extract was found to be homogeneous by TLC and after crystallisation from ethyl acetate-hexane had melting point 116-117°.

The product melting point 111° (heraclenin (LXXIV) is the major component of the mixture and was investigated first.

Heraclenin:

Heraclenin (LXXIV) m.p. 111° was obtained in a yield of 1.8% from the roots of Heracleum candicans. Preliminary examination of the compound showed it to be optically active, $[\alpha]_D^{32}$ =+22 (pyridine), sparingly soluble in cold and completely soluble in warm aqueous alkali. This, along with the absence of any ferric chloride colouration, suggested that the compound was a lactone. The alkaline solutions of the compound had an intense yellow colour which has previously been reported as a characteristic property of compounds possessing a coumarin nucleus.[6] Heracleum candicans belongs to the natural order Umbelliferae and members of this natural order, specially those classified in the genera Heracleum invariably produce furocoumarins. The foregoing tests and the spectroscopic and subsequent degradative evidence showed that the above compound too was a derivative of the linear furocoumarin. The optical data such as infrared, ultraviolet and NMR spectra of the compound were in agreement with a



furocoumarin structure. Coumarins usually exhibit three maxima in the ultraviolet spectra, at 210-20, 245-265 and 295-315 mμ. Ultraviolet absorption curve (Fig. 2) of heraclenin (LXXIV) was almost superimposable on that of imperatorin (LXXX). Ultraviolet absorption fails to distinguish between simple coumarins and furocoumarins, the regions of absorption being almost identical in the two cases, but the infrared spectrum is more informative here. Infrared spectra of a number of furocoumarin have been reported in literature[73] and by comparison of these with those of simple coumarin, the following bands have been assigned to the furan system, 820, 866, 1095, 1620, 3125 and 3175cm^{-1}. Almost all of these bands were found to be present in the infrared spectrum (Fig. 3) of heraclenin (LXXIV), which was again very similar to that of imperatorin and showed further the absence of a hydroxyl band.

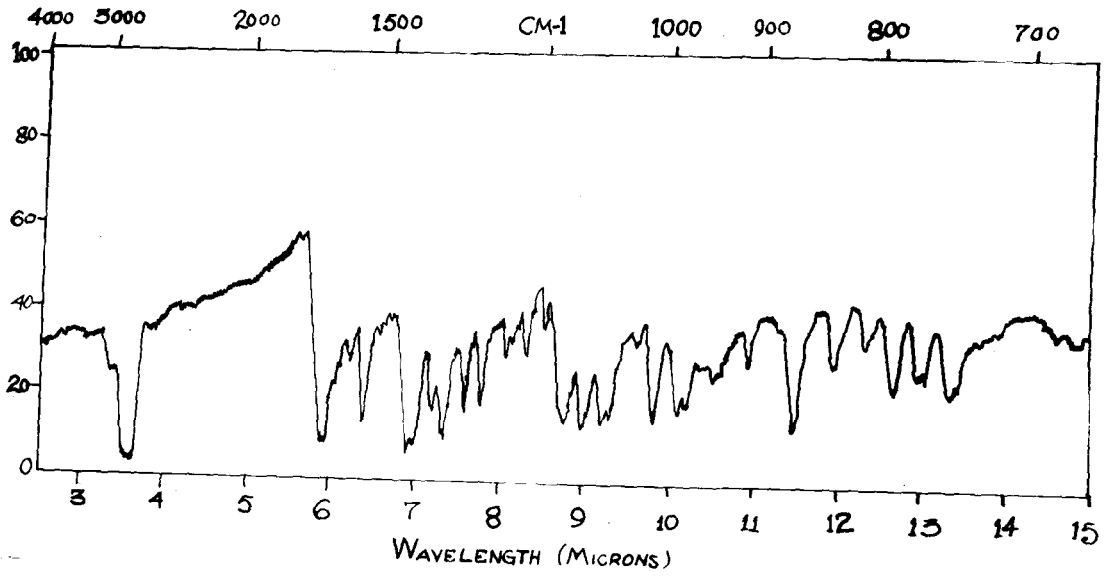


Fig. 3

NMR spectroscopy has been applied more recently to coumarins [74-77] but already sufficient information regarding the chemical shifts of different protons is available and allows assignments of NMR signals to be made with some certainity. NMR spectrum of heraclenin (Fig. 4) shows a doublet where the methyl protons occur (9.0 τ). The doublet is due to different chemical shifts produced by a different steric arrangement of the two methyl groups around the epoxide ring and not due to one proton splitting by the methine proton of an isopropyl group, as the doublet of the latter is not of equal intensity whereas here the two signals are of equal intensity. Besides the methine proton of the isopropyl group should give rise to a multiplet not present in the spectrum of heraclenin. This indicated the absence of a methine proton and suggested that the

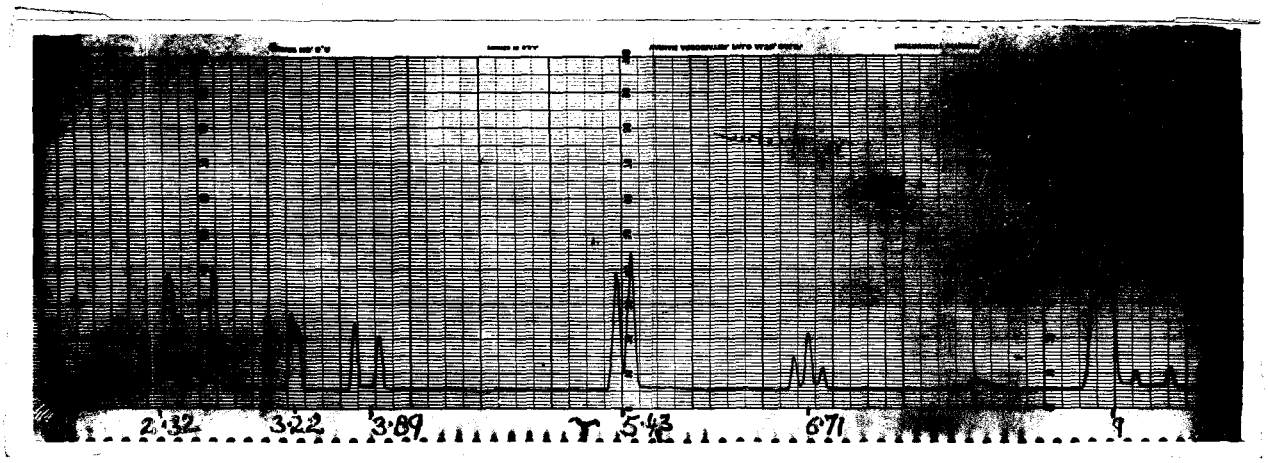
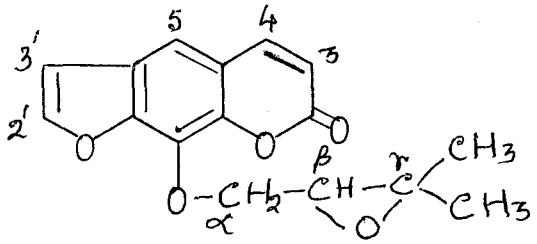


Fig. 4

epoxide ring is present between the β , γ carbon atoms. The β-proton (CH of the side chain) gives rise to a triplet (6.71 τ) and two α-protons (CH_2 of the side chain next to the oxygen atom) to a doublet (5.43 τ). The remaining signals occurring further downfield are due to protons of



LXXIV

the lactone, benzene and furan rings. Out of these the doublet at 2.32 τ can be assigned to one proton at 4, the doublet at 3.89 τ to proton at 3, and the doublet at 3.22 τ to proton at 3', which is in analogue with the signals of this proton in oroselol. The signals for proton at 2' merge with the doublet for the proton at 5, showing a small inflexion due to absorption of the solvent.

In furocoumarins substituents are usually located either at position 5 or 8. There are also examples of furocoumarins which are substituted in the furan ring but of these very few are known.

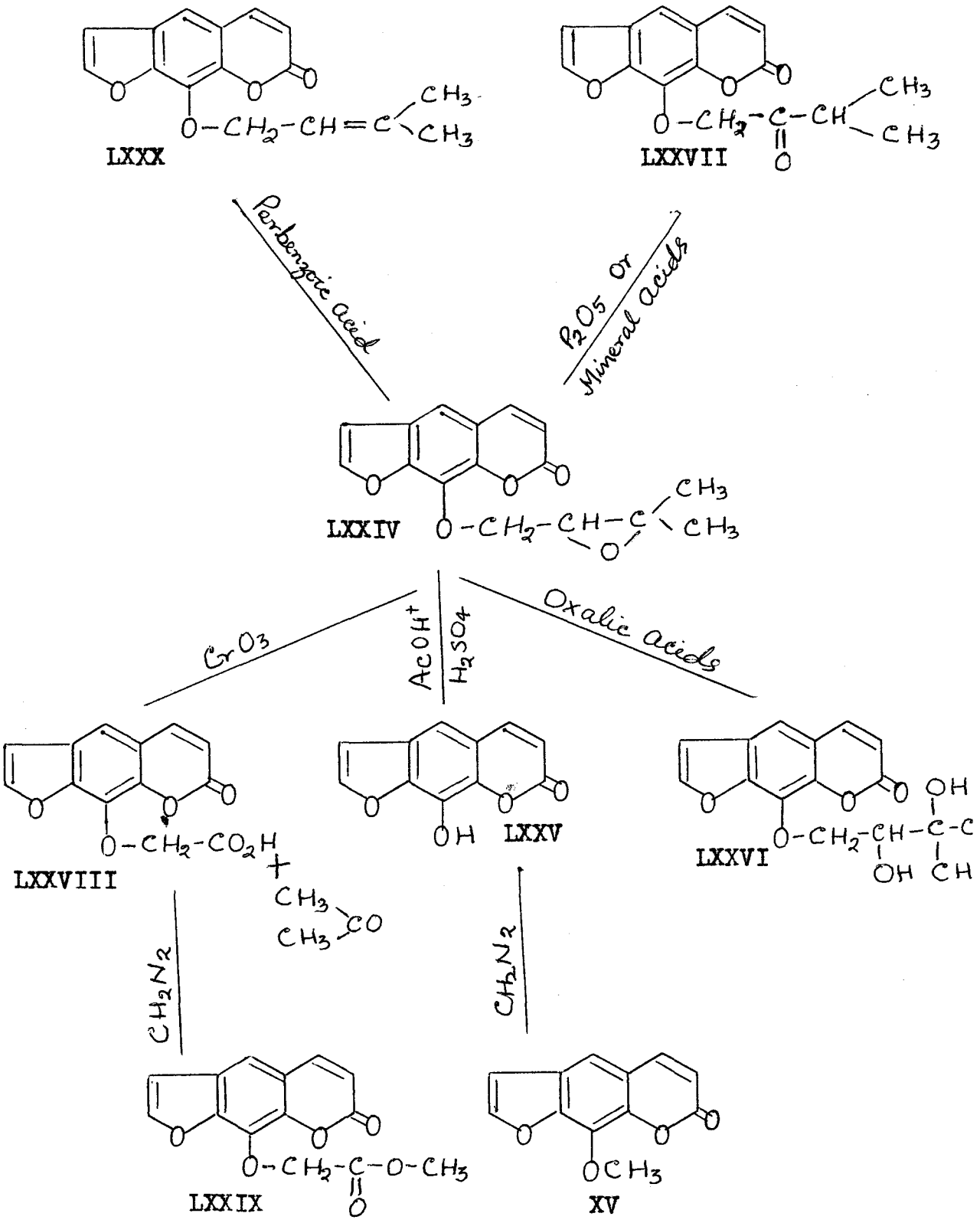
The coumarino-coumarones of the type of wedelolactone, psoralidin belong to a more complex group of naturally occurring compounds containing a coumarino-coumarone nucleus. Compounds of this type have been isolated from several plants belonging to different families and are not characteristic of the natural order umbelliferae.

Heraclenin (LXXIV) analysed for $C_{16}H_{14}O_5$ and showed the absence of both active hydrogen and methoxy groups. With the evidence already cited for the presence of a furocoumarin nucleus, this establishes the nature of three of the oxygen functions. A fourth can be assigned to the ether function, which usually provides the linkage with the side chain. The side chain itself should then be a C_5H_9 one, and it should also contain the remaining oxygen atom. In the absence of active hydrogen and methoxy as shown by analytical data, the oxygen can only be present in an oxiran ring as indicated by the NMR spectrum. Chemical studies to confirm these features were carried out largely on the same lines as were adopted by Späth[1] in his work on similar furocoumarins. This consisted of cleaving the ether linkage with the side chain and examining the substitution of the coumarin nucleus by further oxidative degradation. Späth also described the behaviour of coumarins containing an oxiran ring present in the side chain which was employed to establish with certainity the presence of this ring in heraclenin.

For the cleavage of the ether linkage glacial acetic acid containing a few drops of concentrated sulphuric acid was used, and this gave good results provided the reaction mixture was not overheated. In the case of heraclenin, heating on a water bath for 45 minutes proved quite sufficient, and the cleavage product could be isolated and crystallised easily. Heating the reaction mixture to 130°

as was done by Späth in some cases resulted in extensive charring and crystalline products could not be isolated. Treatment as above led to the isolation of xanthotoxol (LXXV), but it was found that the isolation and crystallisation of this compound was much easier, if the crude product was directly acetylated or methylated to acetyl xanthotoxol or xanthotoxin (XV) respectively. The identity of these products was established by comparison with authentic samples. The nature of C_5 residue had to be determined in the intact molecule itself and the same experiments as were carried out on oxypeucedanin and byakanglicol by Späth and co-workers and Noguchi et al[78] were found to provide the necessary evidence for the presence of an epoxide ring between the β,γ carbon atoms of the C_5 side chain. Thus it was found that treatment with dilute aqueous oxalic acid solution at water bath temperature gives rise to a diol (LXXVI) $C_{16}H_{16}O_6$ m.p. 117°. An attempt was made to convert this to the corresponding acetate but unlike the diol obtained from oxypeucedanin by Späth, the diol from heraclenin did not form a crystalline acetyl derivative.

With dilute mineral acids heraclenin isomerised to a ketone which has been named isoheraclenin (LXXVII) in accordance with the nomenclature adopted by Späth in case of the ketone obtained from oxypeucedanin. Isoheraclenin (LXXVII) analysed for the theoretical value $C_{16}H_{14}O_5$ and had


LXXX
LXXVII
Perbenzoic acid
P2O5 or Mineral acids
LXXIV
CrO3
AcOH+ H2SO4
Oxalic acids
LXXVIII
LXXV
LXXVI
CH2N2
CH2N2
LXXIX
XV

a doublet in the carbonyl region in its infrared spectrum. It also formed a 2,4-dinitrophenylhydrazone.

Presence of a terminal isopropyl group was further established by oxidation of heraclenin (LXXIV) with chromium trioxide when acetone was formed and could be identified as its 2,4-dinitrophenylhydrazone derivative. The second product of the oxidation 8-ω-carboxymethoxy 4',5',6,7-furocoumarin (LXXVIII) could be isolated from the reaction mixture only as its methyl ester (LXXIX) which was readily formed with diazomethane and gave the required $C_{13}H_8O_6$ analytical values. This acid had previously been synthesised by Schönberg and Sina[79] who reported a melting point of 210° for it. The compound obtained by us melted at 215°. A comparison with their material has not been possible.

The structure of this compound was further confirmed by infrared comparison with oxyimperatorin, synthesised according to the procedure of Späth, who synthesised both oxyimperatorin and oxypeucedanin, in connection with the investigation of masterwort coumarins.[80]

While this work was in progress presence of this furocoumarin in Prongas pabularia was reported by Russian workers.[81] The reported melting point of their product is 3° higher than that of heraclenin and identical with the melting point of oxyimperatorin prepared synthetically with perbenzoic acid.

8-Geranoxypsoralen:

The two products melting point 53-54° and 82-83°, hereafter referred to as A (LXXXI) and B respectively, were obtained in comparatively smaller amounts, the former only in traces, from the petroleum ether extract. Both gave typical colour reactions of furocoumarins and the ultraviolet spectra of the two showed absorption in regions associated with furocoumarins. The ultraviolet spectrum of the lower melting product A (LXXXI) showed absorption at 215 ($\log \epsilon$ 4.51) 248 ($\log \epsilon$ 4.42) and 298 mμ ($\log \epsilon$ 4.43); that of the higher melting product B at 217 ($\log \epsilon$ 4.47) 248 ($\log \epsilon$ 4.42) and 298 mμ ($\log \epsilon$ 4.05). Infrared spectra (Figs. 5 and 6) of the two products were almost superimposable, the only marked difference being the absence of 10.6μ band in the spectrum of B. As against this close resemblance in their spectroscopic properties solubilities of the two products differed

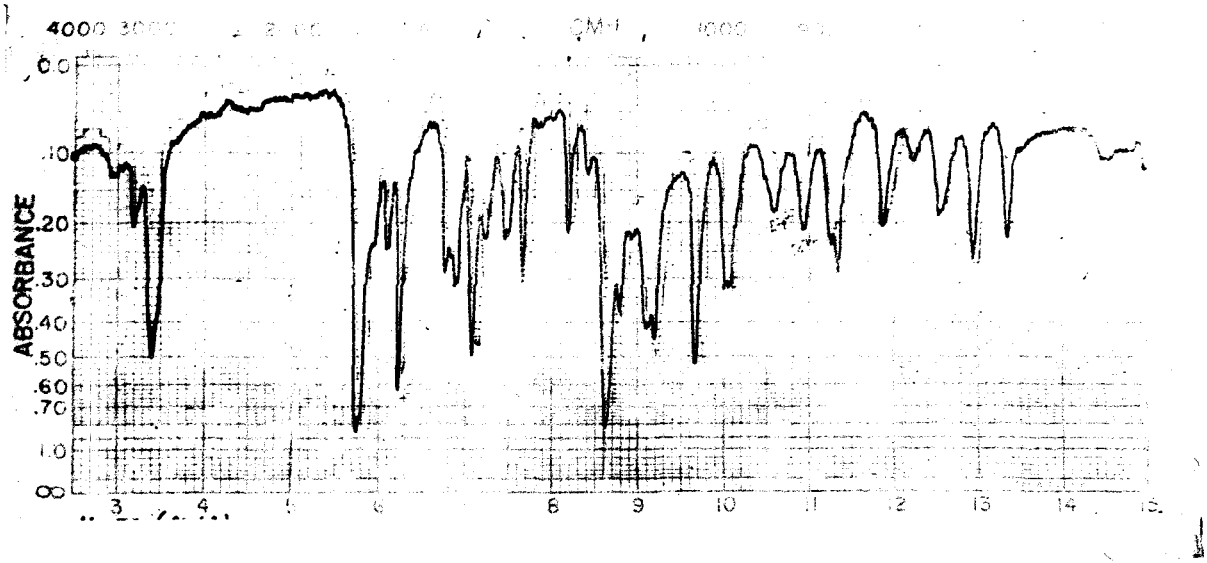


Fig. 5

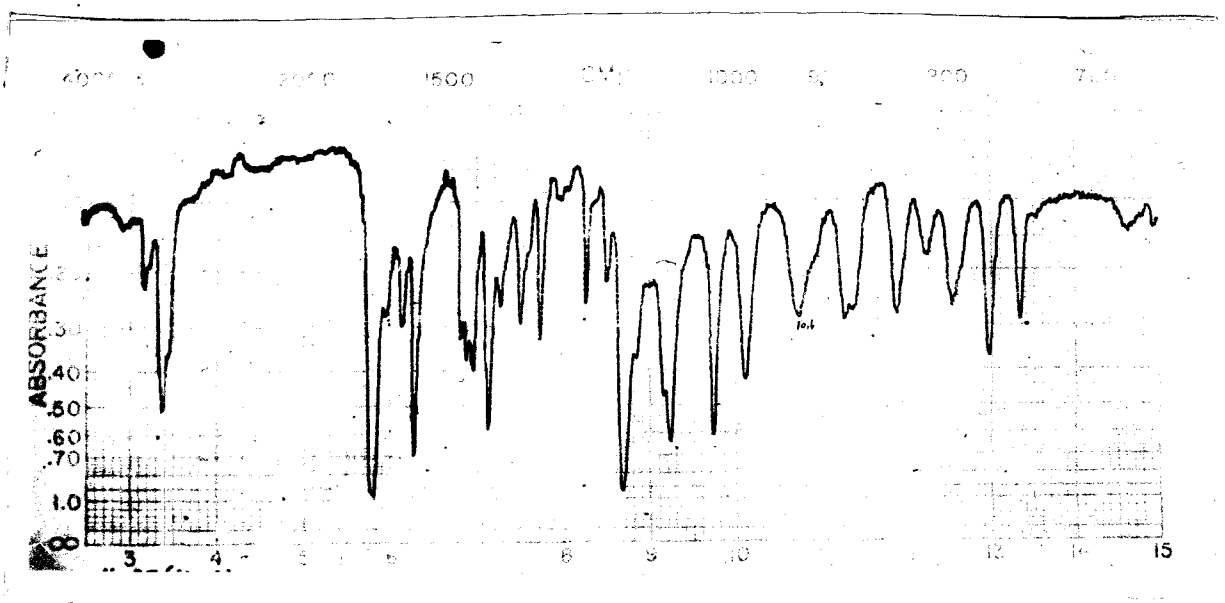


Fig. 6

considerably, A being soluble in petroleum ether from which it was crystallised, while B was insoluble in petroleum ether and was crystallised from methanol.

Analysis of A (LXXXI) agreed with the formula $C_{21}H_{24}O_4$ indicating the presence of a $C_{10}H_{18}$ side chain but the analytical values for B could not be fitted into a reasonable formula. Repeated elementary analysis gave more or less identical values thus ruling out any experimental error in analysis, the results of two determinations being as follows:

(i) C, 72.02; H, 5.86

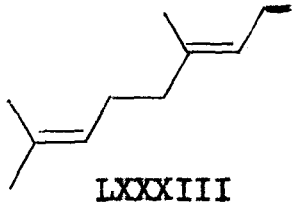
(ii) C, 72.18; H, 5.82

These values work out for an empirical formula $C_{22}H_{21}O_5$, increasing hydrogen by one to get an even number of hydrogen atoms $C_{22}H_{22}O_5$ requires: C, 72.11; H, 6.05, which is in fair agreement with the above values for carbon and hydrogen.

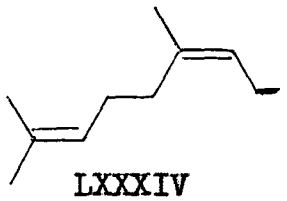
Methoxyl and active hydrogen determinations were negative and the absence of hydroxyl function was also evident from the infrared spectrum. The close similarity between the two products, further emphasised by subsequent degradations, suggested strongly that both the products were derivatives of psoralen with a C_{10} side chain but leaves an extra carbon atom and an oxygen function still to be accounted for in B. The extra oxygen in B could possibly be present in an oxiran ring, however, there is no way of accounting for the excess carbon. This made it very likely that B was contaminated with some impurity even though the melting point of the product was very sharp and did not show any variation on repeated crystallisations. Thin layer chromatography using a number of solvent systems failed to bring about any separation and both products A and B ran side by side on chromatostrips.

Cleavage of the side chain in A and B could be effected easily by treatment with mineral or organic acids at water bath temperature and gave in both cases xanthotoxol and a fragrant oil. The latter was identified by vapour phase chromatography, and through its 3,5-dinitrobenzoate, as geraniol. Ozonolysis of both A and B gave acetone and leavulinic aldehyde (LXXXII) which rules out any double bond migration under the conditions of cleavage reaction and provides confirmation for the presence of a geranyl side chain in both the products. Isolation of identical cleavage

products from A and B can be explained either by assuming the presence of some impurity in one of the products or by the existence of cis-trans isomerism, which is to say that the side chain is attached as in LXXXIII or LXXXIV and the nerol formed from LXXXIV undergoes immediate rearrangement to geraniol during the cleavage reaction. This would have been in keeping with the close resemblance of infrared and ultraviolet spectra of the two products and the variation in the elementary analysis of B from the required values would then be due to the presence of traces of some impurities.



LXXXIII

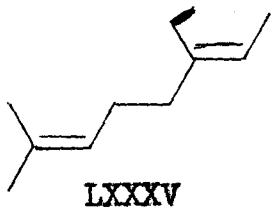


LXXXIV

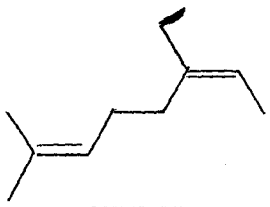
8-Geranoxypsoralen (LXXXI) has been previously reported in literature by W.L.Stanley and co-workers[4], however, these authors have not suggested any reason for assuming the presence of a geranyl in preferance to a neryl side chain. Besides they have assumed the presence of a geranyl side chain only on the basis of analytical values and isolation of xanthotoxol and they failed to get any leavulinic aldehyde (LXXXII) on ozonolysis. The melting point of the product reported by them was 61-62^{o} but the sample kindly provided by them had melting point 53-54^{o}in hand and showed no depression on mixed melting point determination with A.

The identity of the two compounds was further established by infrared comparison of their spectra but the exact nature of the compound isolated by Stanley had still to be determined.

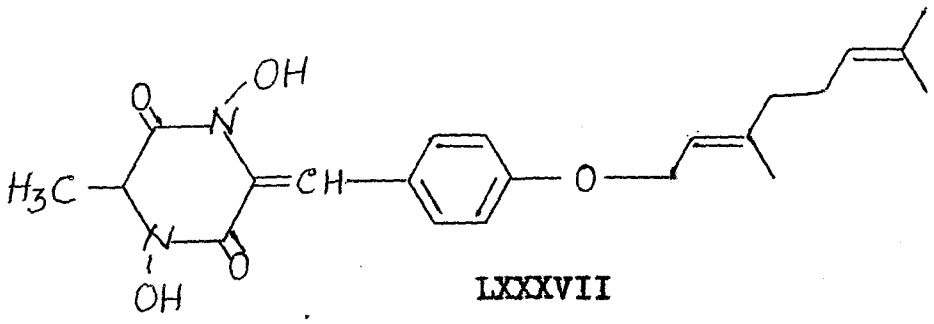
Recently Bates and co-workers[82] re-examined the nature of the side chain in the antibiotic mycelianamide, obtained from Penicillium griseofulvum. NMR spectroscopy indicated that the side chain was as in LXXXIII or LXXXIV rather than as in LXXXV or LXXXVI as originally suggested on the basis of Birch reduction[83] and isolation of acetaldehyde on ozonolysis.[84] Synthesis of the two possible geometrical isomers under stereospecific conditions finally confirmed the structure (LXXXVII) shown below:



LXXXV



LXXXVI



LXXXVII

A similar procedure was adopted to clarify the nature of the two products here isolated. NMR spectrum of 'A' (Fig. 7) had all the signals expected of a psoralen derivative with a C_{10} side chain. The spectrum of 'B' (Fig. 8)

on the other hand showed certain discrepencies which could be accounted for only on the assumption that 'B' was contaminated with certain amount of a psoralen derivative

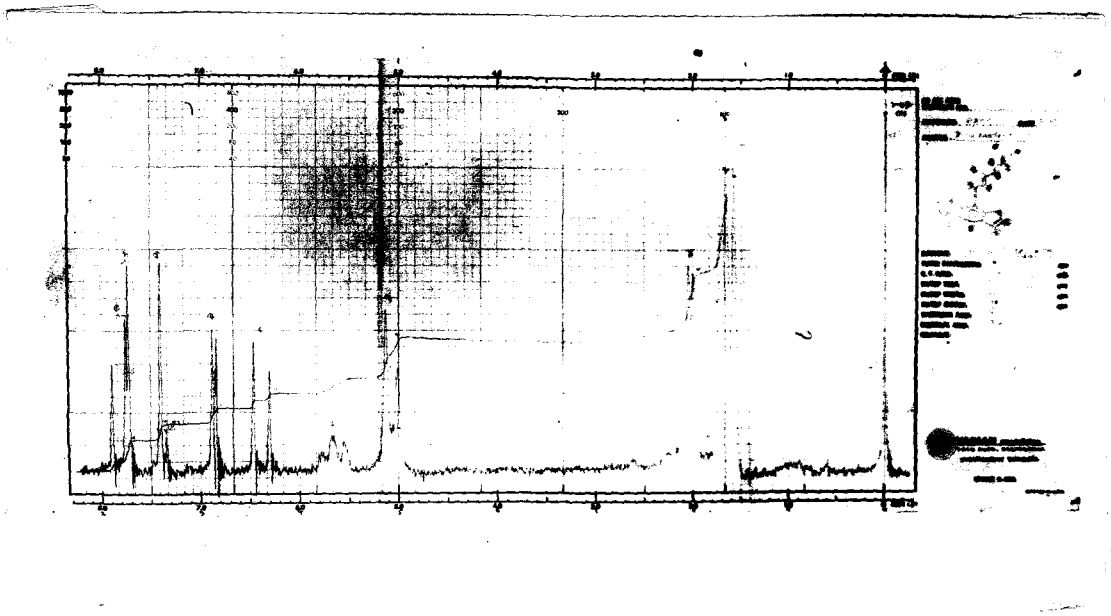


Fig. 7

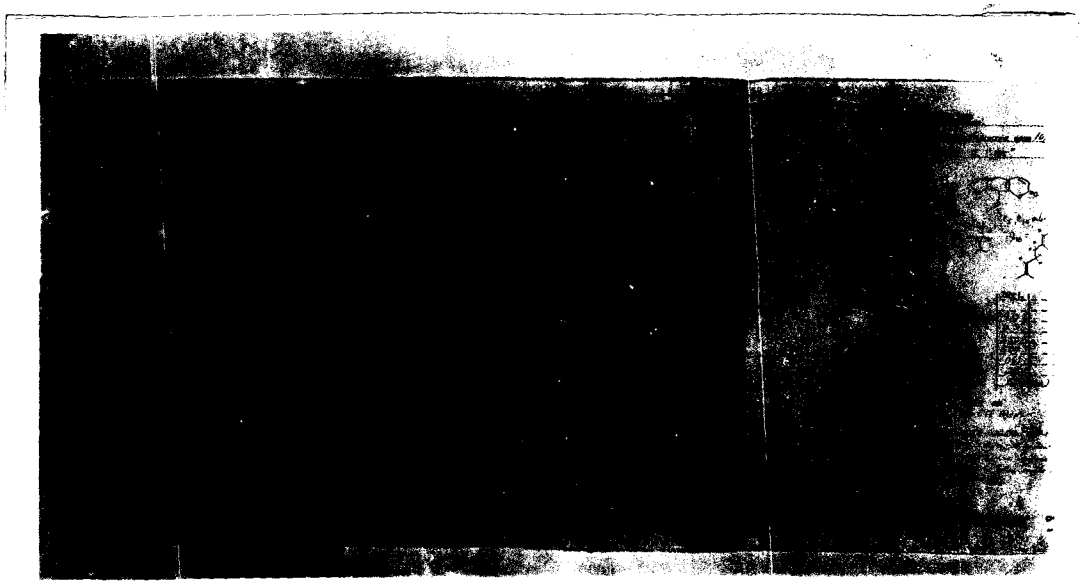
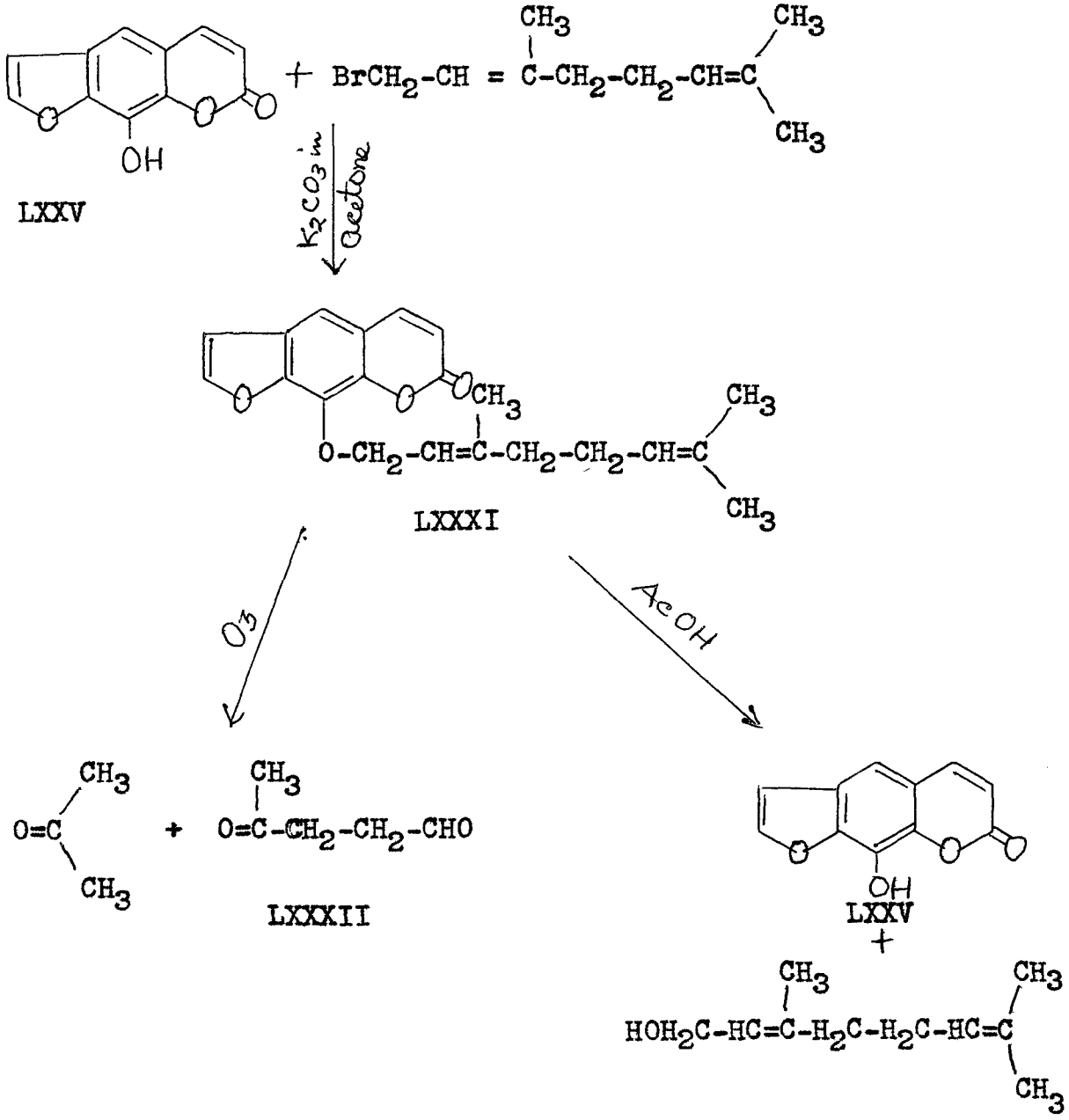


Fig. 8

having a C_5 side chain. The NMR spectrum of 'B' showed a doublet at 4.82 τ due to $O-CH_2-C=C$ protons which would be expected from both C_5 and C_{10} compounds but absorption at 7.91 τ can arise only from the methylene protons ($C=C-CH_2-CH_2-C=C$) of a C_{10} compound.The integrated intensities of the two peaks show a 2:1 ratio in favour of $O-CH_2-C=C$ protons indicating that the side chain must consist of three times as much C_5 as C_{10}. The C_5 component can only be imperatorin (LXXX) because degradation of this would also give rise to the same phenol, xanthotoxol (LXXV). This was confirmed by preparing such a mixture of the two compounds (A + imperatorin) which had the exact melting point of the product 'B' and showed no depression in melting point on admixture with it. It was further noticed that such a mixture does not separate on chromatostrips and otherwise behaves as a single entity. This was finally confirmed by Birch reduction[83] of 'B' which gave 2-methyl-2-butene and <u>trans</u>-2,6-dimethyl-2,6-octadiene (methyl-geraniolene), identified by VPC. These findings were also confirmed by a synthesis of 8-geranoxypsoralen(LXXXI). This was at first attempted by the procedure employed by Chatterjee and Chaudhary[85] for the synthesis of bergamottin but this method did not succeed. It could, however, be synthesised by the method of Schonberg and Sina[79] by refluxing xanthotoxol (LXXV), geranyl bromide in dry acetone over


OH
LXXV
+ BrCH2-CH = C(CH3)-CH2-CH2-CH=C(CH3)2
K2CO3 in acetone
O-CH2-CH=C(CH3)-CH2-CH2-CH=C(CH3)2
LXXXI
O3
AcOH
O=C(CH3)2
+
O=C(CH3)-CH2-CH2-CHO
LXXXII
OH
LXXV
+
HOH2C-HC=C(CH3)-H2C-H2C-HC=C(CH3)2

anhydrous potassium carbonate. The product after purification gave no depression in melting point on admixture with A (LXXXI).

This proved unambiguously that the product 'B' is a mixture of 'A' with imperatorin (LXXX) and that 'A' itself is 8-geranoxypsoralen (LXXXI). Isolation of 'B' is not the result of a chance separation as chromatography of the crude coumarin mixture consistently gave 8-geranoxypsoralen on elution with petroleum ether, and the above mixture 'B' of imperatorin and 8-geranoxypsoralen LXXXI with petroleum ether benzene 5:1 in the ratio already mentioned. Had this been the result of a simple retention of some 8-geranoxypsoralen (LXXXI) on the column, which is then subsequently washed down along with imperatorin contained in the coumarin mixture, a definite ratio between the quantities of two products would not have been the case. It is possible that imperatorin forms some sort of a complex mixture with a fixed amount of 8-geranoxypsoralen (LXXXI).

Coumarins from the Benzene extract:

Heraclenol (LXXVI) m.p. 116-117°was obtained from the benzene extract of the roots, had the characteristic coumarin properties, gave intense yellow colour with alkali and a wine red colour with concentrated sulphuric acid. The ultraviolet spectrum (Fig. 9), $\lambda^{Ethanol}_{max.}$ 249(log ϵ 4.24), 305 (log ϵ 4.0) and 320 mμ(log ϵ 3.88) was again very similar to that of imperatorin (LXXX) suggesting it to be furocoumarin derivative.

Empirical formula of heraclenol (LXXVI) corresponds to $C_{16}H_{16}O_6$, indicating that it has one oxygen atom more than heraclenin (LXXIV). It did not form an acetate and gave no colouration with ferric chloride but the infrared spectrum (Fig. 10) clearly showed the presence of the hydroxyl band

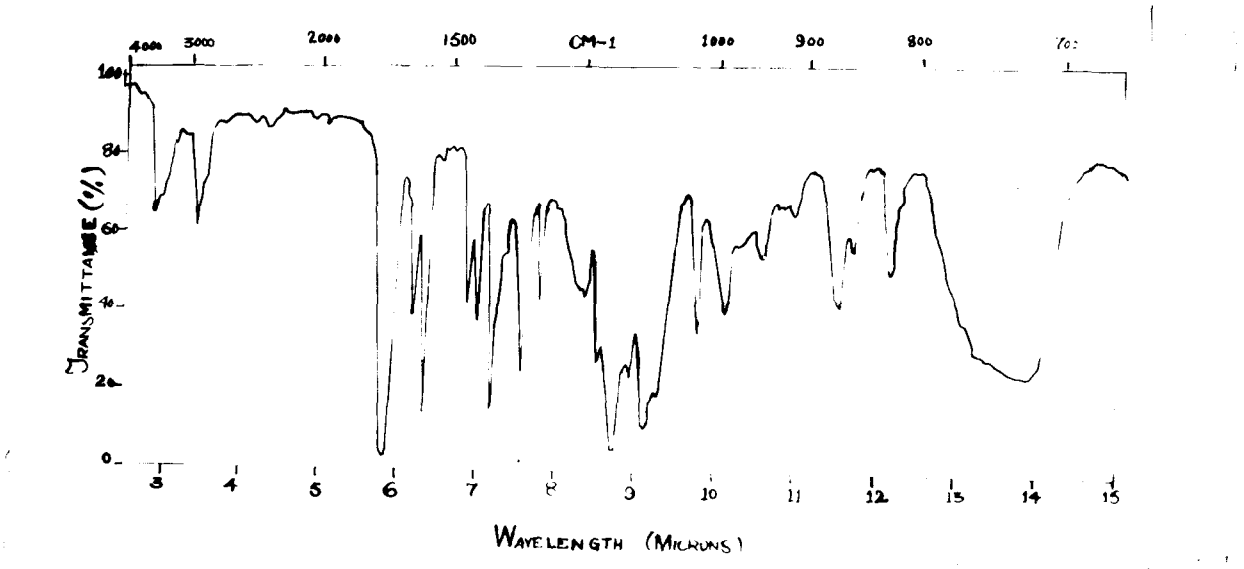


Fig. 9

at 2.9 mμ. Cleavage of the compound as in the case of heraclenin gave xanthotoxol (LXXV), identified by comparison with an authentic sample and ruled out the possibility of substitution in the nucleus. Both the oxygen atoms were thus shown to be present in the side chain and one of them at least as a hydroxyl function. From considerations of its co-occurrence with heraclenin, it appeared very likely

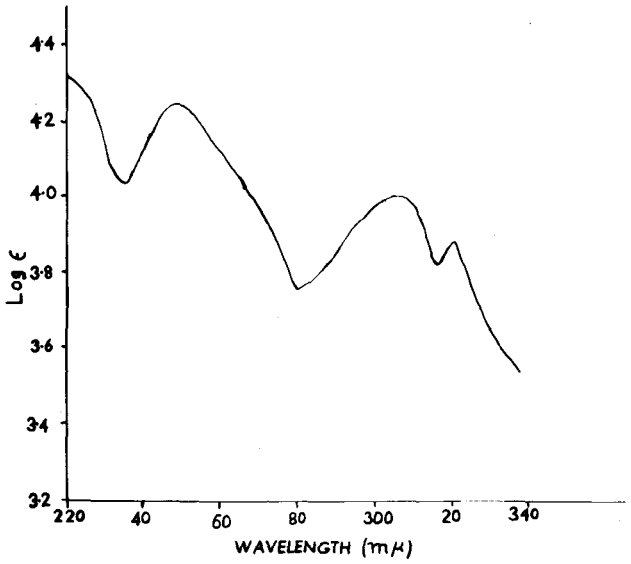


Fig. 10

that the epoxide ring of the latter had been cleaved here to give rise to a dihydroxy compound. This was confirmed by treatment of heraclenol with phosphorous pentoxide in toluene solution when a ketone (LXXVII) identical in melting point with the one obtained from heraclenin was obtained. Further, the dihydroxy compound (LXXVI) formed when heraclenin was treated with oxalic acid did not depress the melting point of heraclenol and this definitely confirmed its structure as 8-(β,γ-dihydroxyisoamyloxy)-psoralen.

Seseli sibiricum:

Seseli sibiricum Benth[71] is a shrub growing widely in the higher mountaineous regions of North Western Himalays. The plant collected from Jammu and Kashmir is locally used for a variety of skin ailments under the name of Bhootkeshi.

Prolonged extraction of air-dried roots of Seseli sibiricum with petroleum ether yielded an oily material with strong odour of essential oils. As a detailed investigation of the volatile constituents of this fraction had already been reported by Handa and co-workers[86], an attempt was made to isolate any cyrstalline constituents from the plant extracts. For this purpose the oily material was chromatographed over alumina, on eluting the column with petroleum ether again an oily material was obtained but this when kept in ice-chest for several days deposited colourless crystals which could be recrystallised from petroleum ether melting point 84-85°. The ultraviolet spectrum[87] (Fig. 11) of this compound showed maxima in the region where coumarins are known to absorb and in other properties also it resembled coumarins.

The compound analysed for $C_{15}H_{16}O_3$ and one methoxyl, indicating the presence of an isopentenyl side chain which could not, however, be cleaved in the normal manner and appeared to be directly joined to the ring carbon atom. This suggested that compound could be osthol melting

point 84-85°, which was further confirmed by oxidation[88] to an acid melting point 253-54°, which agreed well with the reported melting point of ostholic acid, acetone obtained alongside was identified as its 2,4-dinitrophenylhydrazone. Further just like ostholic acid this acid could be decarboxylated to 8-methyl-7-methoxy coumarin melting point 136-37°.

The identity of coumarin with osthol was established by comparison with an authentic sample obtained from the Regional Research Laboratory, Jammu.

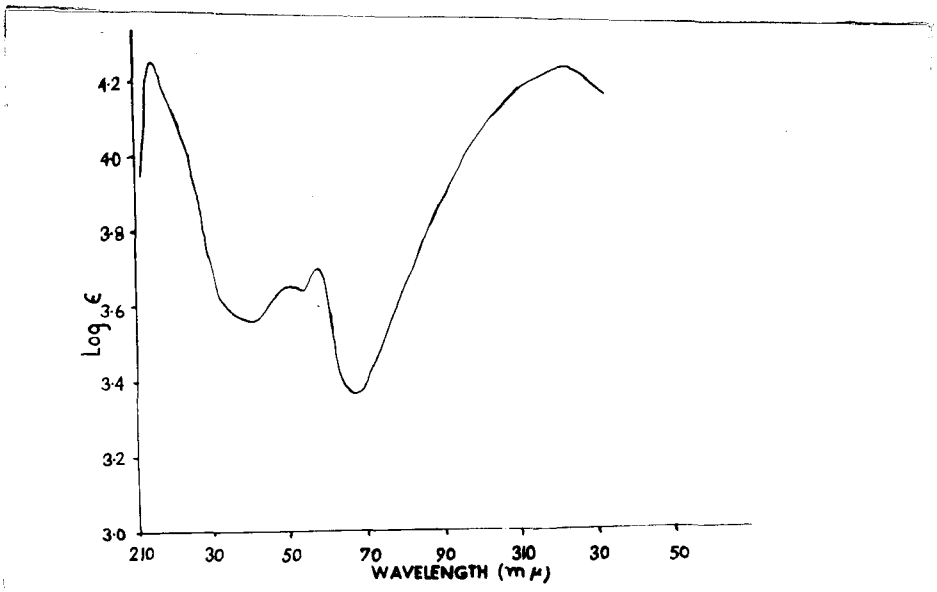


Fig. 11

Fenchyl p-hydroxy cinnamate:

When the petroleum ether eluate on evaporation to dryness showed the absence of essential oils, the column was eluted with benzene. The benzene fraction on evaporation to dryness left a viscous mass which was crystallised from methanol melting point 215-16°. It was found to be present in the plant to the extent of 0.1 per cent. It analysed for $C_{19}H_{24}O_3$, was optically inactive, insoluble in alkali and did not give the characteristic colour of coumarins with alkali. The ultraviolet spectrum (Fig. 12) had λ max. 230(log ε 4.19) and 320 mμ(log ε 4.45) which also indicated that the compound was not coumarinic in nature. But as the ultraviolet spectrum compared favourably well with that of p-hydroxycinnamic acid[89] and the infrared

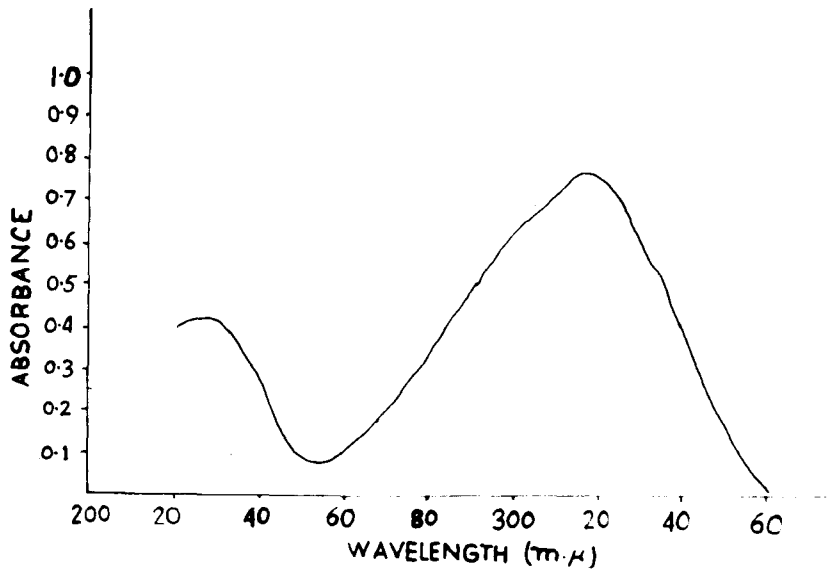


Fig. 12

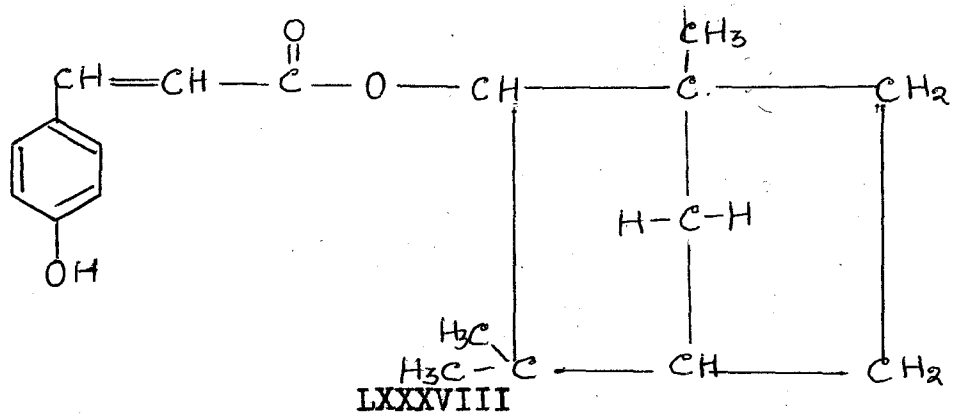
spectrum showed distinctly the presence of an ester carbonyl, it seemed likely that the compound was a derivative of p-hydroxycinnamic acid. Further as the infrared spectrum also showed the presence of hydroxyl group, the compound could only be an ester of p-hydroxycinnamic acid and not joined to the alcohol by ether linkage, with ferric chloride the compound gave green colouration, it formed an acetate melting point 95° and also reacted with ethereal solution of diazomethane but the methyl ether could not be induced to crystallisation, however, it showed that the compound contains a free hydroxyl group.

On slightly heating with dilute alkali it decomposed giving rise to a camphor like odour. The same odour was observed on treatment with dilute acids. The compound was, therefore, taken up in dilute methanolic potash and warmed for some hours on a water bath, the methanol evaporated in vacuum and the residue taken up in water. The aqueous extract was extracted with ether and then acidified and again extracted with ether. From the first ether extract on evaporation an oily, slightly coloured substance was obtained which could not be crystallised but as it had a distinct odour of camphor it seemed likely that the compound belonged to this class. It was distilled in vacuum when a few drops of colourless oil were obtained which solidified on keeping in an ice-chest to give needles melting point 35-40°. It formed a crystalline p-nitrobenzoate melting

point 107-109° which corresponded with the melting point of fenchyl p-nitrobenzoate.[90] Its identity was finally confirmed by comparison of its infrared spectrum with the spectrum of an authentic sample of fenchyl p-nitrobenzoate.

The second ether extract afforded a solid which was crystallised from petroleum ether and then sublimed under vacuum, melting point 210-212°. It formed an acetate melting point 208-210° and methyl ether melting point 175°. These melting points were in agreement with p-hydroxy-cinnamic acid[91] and its corresponding derivatives. The identity was further confirmed by comparison with an authentic sample.

The compound was thus shown to have the following structure which was further confirmed by its synthesis from p-acetoxycinnamoyl chloride and fenchyl alcohol.



LXXXVIII

p-Hydroxycinnamoyl chloride itself did not react with fenchyl alcohol in the desired manner and appeared to have undergone intermolecular condensations giving rise to polymeric products.

The co-occurrence of fenchyl p-hydroxycinnamate, osthol and other essential oils in Seseli sibiricum is an interesting feature biogenetically as esters of monoterpenic alcohols with cinnamic acid have not been reported so far.

Angelica glauca Edgw:

Angelica glauca Edgw locally called Chora or Chura[92] is a shrub growing in the higher regions of Kashmir. It is used locally as a cardiac stimulant and in the treatment of dyspepsia and constipation. The aromatic root is a flavouring agent.

Though other members of the genera Angelica have been investigated both in India and abroad, this particular specie has so far received scant attention.[93] The plant occurs abundantly, but is scattered over wide areas and the ripening season of the fruits is short. Only a small quantity of the seeds could, therefore, be collected for the present investigation.

Extraction with hexane and concentration of the extract gave a dark green solid which was dissolved in ether and freed from acidic impurities by extraction with sodium bicarbonate. Evaporation of the solvent and crystallisation of the residue from ether-hexane mixture gave yellow crystals melting point 90-105°.

The above solid was found to be inhomogeneous on thin layer chromatography[2] (Fig. 13). Separation was effected over a column of alumina and afforded three crystalline products melting points 109-110°, 103-104° and 119-120°. The first two were identified as isoimperatorin (LXXXIX) and prangolarin (XC).[94] Isoimperatorin has already been reported from other members of this genera but prangolarin

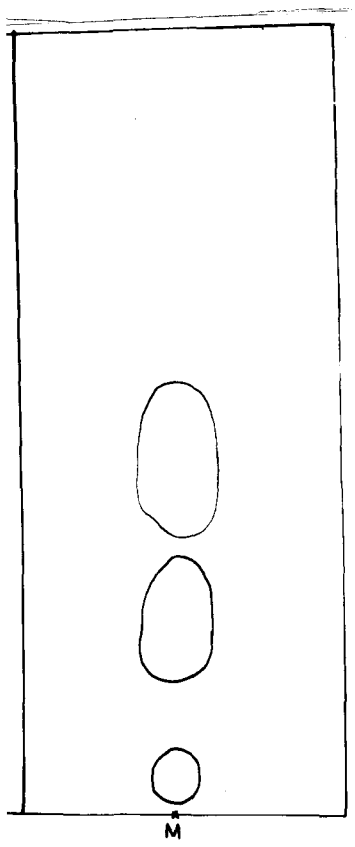
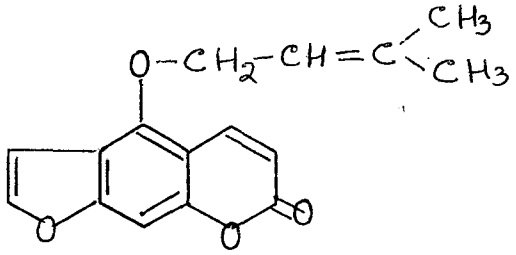
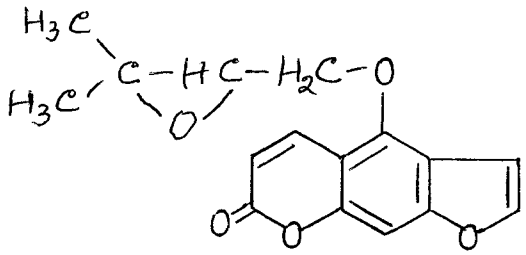


Fig. 13

has only recently been isolated from Prangos pabularia by A. Chatterjee et al.[6] The third compound forms less than 7% of the total coumarin mixture and in all about 400 mg. of the purified material could be collected.



LXXXIX



XC

Elementary analysis agreed with the formula $C_{17}H_{18}O_6$ Methoxyl estimation showed further the presence of one methoxyl. A coumarin of the above formula having the same

melting point has so far not been reported and the compound thus appeared to be new. The quantity of the compound was not sufficient for chemical degradation and the proposed structure is based on its ultraviolet, infrared, NMR and mass spectra. The ultraviolet spectrum (Fig. 14) was

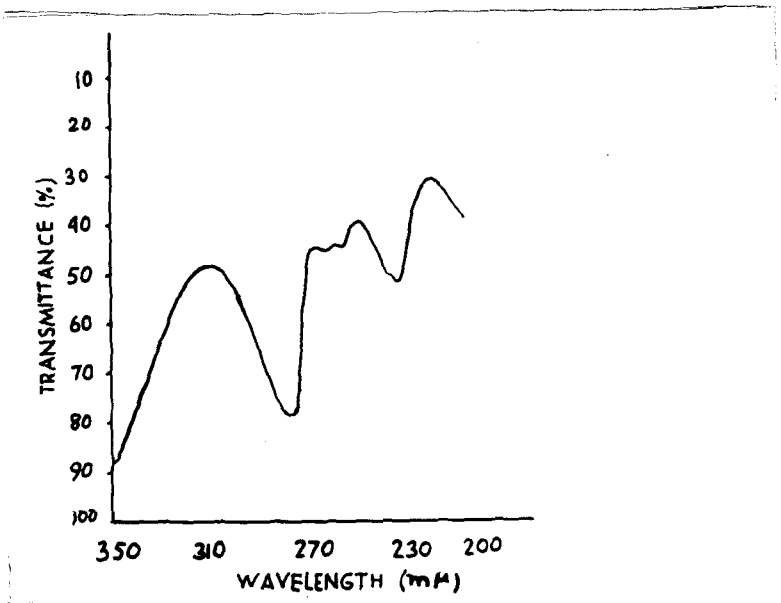


Fig. 14

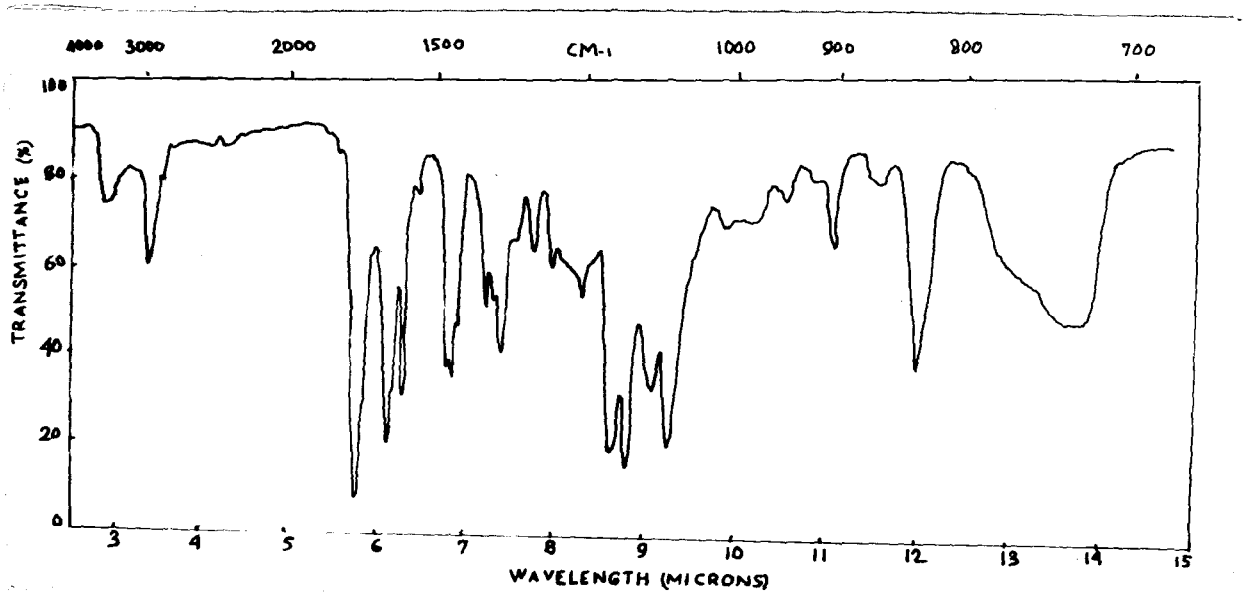


Fig. 15

characteristic of 5,8-disubstituted furocoumarins having absorption at 222,250,268 and 310 mμ. The infrared spectrum (Fig. 15) clearly showed the presence of hydroxyl band at 2.85μ and δ-lactone (5.75μ). The compound does not form an acetate and hydroxyl thus appears to be tertiary. The NMR spectrum (Fig. 16) is in agreement with a furocoumarins structure substituted both in 5 and 8-positions. A pair of doublet (τ = 1.75 and 3.65; J. 10 c/s) can be assigned to protons (4) and (3) respectively, the signals at 2.4τcan be assigned for proton (2') and at 2.95 τ for (3'). Furthermore the spectrum has signals fof methoxyl and hydroxyl protons. On the

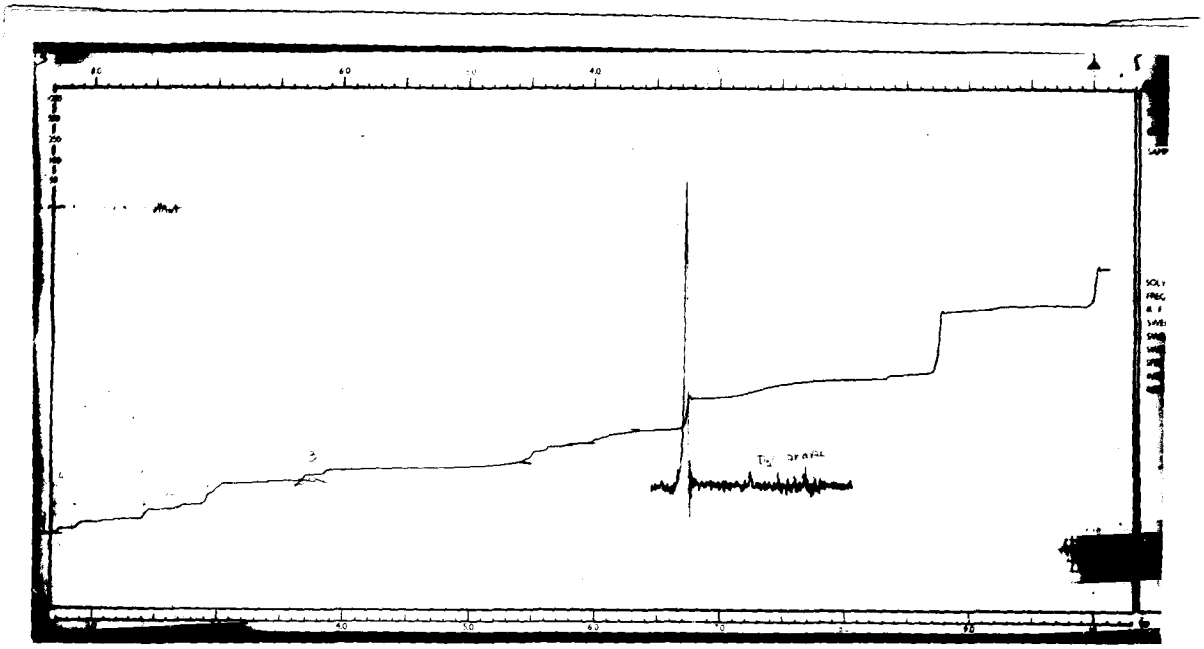
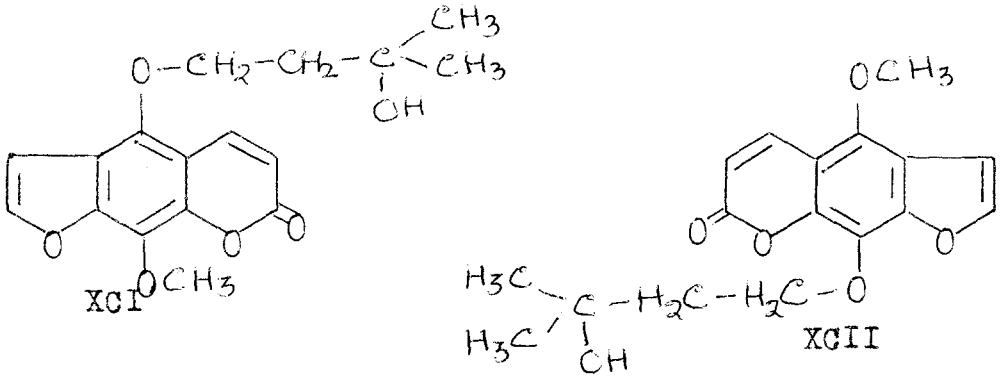


Fig.16

basis of above studies the following two structures XCI and XCII are tentatively assigned to the compound which agree with its mass spectrum fragmentation pattern (Fig.17)



Thus the mass spectrum shows the molecular ion as the base peak (318) and the ions due to loss of CH_3 (303), $(CH_3)_2COH$ (244), $C_5H_{11}O + CH_3$ (202) and $CO + COCH_3$ (174). The rest of the spectrum was very similar to that of 5,8-disubstituted furocoumarins.

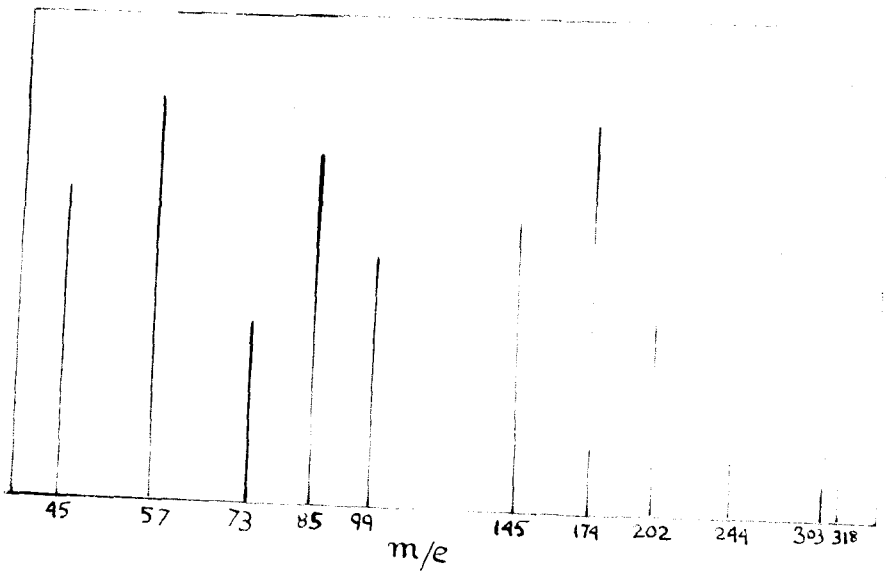


Fig. 17

E X P E R I M E N T A L

EXPERIMENTAL

All ultraviolet spectra were measured on a Beckmann Model DU or DB instrument, either in 95% ethanol or methanol. Infrared spectra were recorded on a Perkin Elmer Infracord, either in chloroform solutions or as mulls in nujol. NMR spectra were recorded at the Chemistry Department, University of Arizona and at the Chemistry Department, University of Liverpool, England on Varian A-60 spectrometer in $CDCl_3$ with tetramethylsilane as internal reference. Mass spectra were also recorded at the Chemistry Department, University of Liverpool England.

All the melting points recorded in this thesis were determined on a Kofler block and are uncorrected.

Preparation of chromatostrips:

Silica gel (250 g.) and starch (5 g.) were made into a slurry with distilled water (250 cc). The slurry was then heated on a boiling water bath for 10 minutes with constant stirring. It was then spread over clean, dry glass plates (20x7 cm) to a thin layer of about 250 μ and the plates were dried in an oven at 110-120^{o} for 45 minutes. The chromatostrips were cooled to the room temperature, and kept at the same temperature for 15 minutes before use.

A spot of the substance to be chromatographed, was applied about 3 cm. from one end of the plate and it is then introduced with the spotted and downward into a chamber containing the developer and previously saturated with the same solvent. The spots were made visible under ultraviolet light.

Deactivation of alumina:

Aluminium oxide (chromatographic) (1 kg.) of E.Merck was deactivated by shaking in a mechanical shaker for two and a half hour with 10% aqueous acetic acid (50 cc).

Purification of Solvents:

1. Petroleum ether:

Petroleum ether usually contains some unsaturated hydrocarbons (chiefly aromatic). These are removed by shaking it two or three times with 10% of the volume of concentrated sulphuric acid; it is then vigorously shaken with successive portions of concentrated solution of potassium permanganatein 10% sulphuric acid untill the colour of permanganate remains unchanged. The solvent is then thoroughly washed with water, dried over anhydrous calcium chloride and then distilled. It is kept dry over sodium wire.

2. Benzene:

Commercial benzene contains thiophene which can not be removed by distillation due to their close boiling points The commercial benzene is shaken repeatedly with 15% of its volume of concentrated sulphuric acid untill the acid layer is colourless or very pale yellow on standing for some time. The benzene is then washed twice with water to remove most of the acid and dried over anhydrous calcium chloride, After filtration the benzene is distilled and the fraction between 80-81^{o} is collected. It is then kept over sodium wire.

3. Pyridine:

It is purified by refluxing over sodium or potassium hydroxide and then distilled with careful exclusion of moisture

and kept over sodium or potassium hydroxide.

4. Chloroform:

The commercial chloroform contains about 1% ethyl alcohol as stabiliser and it is removed by shaking five or six times with half its volume of water, then dried over anhydrous calcium chloride and distilled. Stored in coloured bottles.

5. Ethyl acetate:

Ordinary ethyl acetate usually contains some water, ethyl alcohol and acetic acid. A mixture of ethyl acetate (1 l.) acetic anhydride (100 cc) and concentrated sulphuric acid (10 drops) is refluxed on a water bath for 4 hours and then fractionated. The distillate is then refluxed with anhydrous potassium carbonate for 1 hour and distilled with careful exclusion of moisture stored in a tightly stoppered bottle.

6. Acetone:

The acetone is refluxed with successive small quantities of potassium permanganate untill the violet colour persists. It is then distilled and the distillate refluxed over anhydrous potassium carbonate and then fractionated.

7. Ether:

The technical grade ether is shaken three or four times with large excess of water, and then dried over anhydrous calcium chloride. The ether is then filtered and distilled in a dry clean Winchester bottle and kept over sodium wire.

Heracleum candicans:

Petroleum ether extract:

Powdered air dired roots of Heracleum candicans (5 kg.) were refluxed with petroleum ether (60-80°) 20 litres on a water bath for 20 hours. The extract was then filtered off and the operation repeated twice. The combined extracts on cooling gave (150 g.) a yellowish white solid. The solid was filtered off and the filtrate concentrated under reduced pressure to 2 litres. The concentrated extract on keeping in a refrigerator for three days yielded another crop of 150 g. of a solid. The material thus obtained constituted about 6% of the dry roots.

Separation of the coumarins:

A column (4 x 100 cm) was packed by deactivated alumina (500 g.) made into a slurry with petroleum ether (40-60°) when the column has properly set the crude mixture of coumarins (10 g.) dissolved in minimum amount of benzene was poured on it and the column was then eluted with petroleum ether (40-60°). The eluate on concentration gave an oily residue (250mg) which crystallised from large excess of petroleum ether (40-60°) in stout, colourless needles melting at 50-52°. Further crystallisations from the same solvent raised the melting point to 53-54°.

The column was then eluted with petroleum ether-benzene mixture (5:1) and the eluate, on removal of the solvent, gave a colourless solid mass (500 mg.) melting within a range of

10°. After repeated crystallisation from methanol, it melted at 82-83°.

Final elution of the column with petroleum e ther-benzene mixture (1:1) gave a solid mass (3 g.) melting at 106-109°. It was recrystallised to a constant melting point 111°from methanol.

Heraclenin:

Heraclenin melting point 111°, $(\alpha)_D^{32}$ = + 22 (pyridine), λ max. 250 (logϵ 4.31) and 305 mμ(logϵ 4.02), obtained from the petroleum ether-benzene mixture (1:1) eluate, analysed for the following data.

Analysis: Found C, 67.08; H, 4.99

$C_{16}H_{14}O_5$ requires: C, 67.12; H, 4.93%.

Xanthotoxol:

Heraclenin (1 g.) was dissolved in glacial acetic acid (20 cc) containing concentrated sulphuric acid (20 drops). The reaction mixture was heated on a boiling water bath for 30 minutes, cooled and poured on crushed ice contained in a 250 cc beaker. The gummy product which separated out was sublimed at 190-200°(bath); 1 mm. Crystallisation of the sublimate from methanol gave slightly yellow crystals of xanthotoxol melting point 248-49°.

Analysis: Found: C, 64.98; H, 3.14

Calculated for $C_{11}H_6O_4$; C, 65.35; H, 2.99%

Xanthotoxol acetate:

Xanthotoxol (500 mg.) acetic anhydride (5 cc) and fused sodium acetate (100 mg.) were refluxed together on a sand bath for 1 hour. The mixture was cooled and poured over crushed ice contained in a 250 cc beaker. The solid precipitated was filtered through a Hirsh funnel and washed free of sodium acetate with distilled water. Crystallisation from methanol gave colourless crystals melting point 178°

Analysis: Found: C, 65.62; H, 3.51

Calculated for $C_{13}H_8O_5$: C, 63.94; H, 3.30%

Xanthotoxin:

Xanthotoxol (200 mg.) dissolved in methanol (5 cc) was treated with diazomethane in ether. The reaction mixture was allowed to stand at room temperature overnight. The removal of the solvent gave an oily residue which was dissolved in benzene and chromatographed over deactivated alumina. The benzene eluate on concentration gave yellowish coloured rhombic crystals of xanthotoxin melting point 146-147°.

Analysis: Found: C, 66.27; H, 3.51

Calculated for $C_{12}H_8O_4$: C, 66.67; H, 3.73%.

Xanthotoxin nitrate:

Xanthotoxin (200 mg.) dissolved in glacial acetic acid (2 cc) was treated with concentrated nitric acid (sp.gr. 1.42) (0.5 cc) at room temperature. The reaction mixture was allowed to stand for ½ hour and diluted with water when yellow microcrystalline needles separated out. The nitrate was filtered, washed well with water to remove the last traces of acid and then crystallised from ethanol in bright yellow needles melting at 237°.

Heraclenin hydrate:

A suspension of heraclenin (200 mg.) and water (50 cc) was heated on a water bath for 10 minutes and oxalic acid (50 mg.) was then added and the heating continues for another 10 minutes. The reaction mixture on cooling gradually

deposited a crystalline solid. This was washed free of acid and then crystallised from ethyl acetate to give colourless crystals of heraclenin hydrate melting at 117-118°.

Analysis: Found: C, 63.28; H, 5.39

$C_{16}H_{16}O_6$ requires: C, 63.15; H, 5.30%.

Isoheraclenin:

Heraclenin (1 g.) was dissolved in dry toluene (50 cc) and the mixture was brought to boiling. Phosphorous pentoxide (4 g.) was then added to the boiling solution and heating continued for another 10 minutes. The reaction mixture was cooled, filtered, and the filtrate was diluted with ether (500 cc). The ether-toluene layer was first washed with water, then with 5% aqueous solution of sodium bicarbonate and finally with water and dried over anhydrous sodium sulphate. The solvent was filtered and concentrated to dryness under reduced pressure. The residue was crystallised for ether, melting point 132-134°. It forms a crystalline 2,4-dinitrophenyl hydrazone.

Heraclenin (200 mg.) was heated on a water bath with 10% aqueous sulphuric acid (20 cc) for ½ hour. The reaction mixture was cooled and the solid which separated out was washed with water, dried in a desiccator and crystallised from ether melting point 132-134°.

Analysis: Found: C, 67.33; H, 5.06

$C_{16}H_{14}O_5$ requires: C, 67.12; H, 4.93%.

Heracleninic acid:
(8-ω-carboxymethoxy-4',5',6,7-furocoumarin)

To heraclenin (3 g.) dissolved in glacial acetic acid (45 cc) chromium trioxide (1.2 g.) dissolved in 50% aqueous acetic acid (60 cc) was added and the reaction mixture was allowed to stand at room temperature for 24 hours. It was diluted with water and extracted with a large excess of ether. The ethereal layer was washed well with water and dried over anhydrous sodium sulphate. The solvent was filtered off and concentrated to dryness under reduced pressure. The reddish brown residue thus obtained was dissolved in methanol (10 cc) and treated with diazomethane in ether. The reaction mixture was allowed to stand overnight and the solvent was removed. The methyl ester was dissolved in the minimum amount of benzene and chromatographed over a column of acetic acid deactivated alumina. The benzene eluate gave a yellowish solid which on crystallisation from methanol melted at 146-147°.

Analysis: Found: C, 61.16; H, 3.73

$C_{14}H_{10}O_6$ requires C, 61.32; H, 3.68%.

The above ester (0.3 g.) was refluxed with 50% aqueous acetic acid (16 cc) for 1 hour. The reaction mixture was cooled, diluted with water (35 cc) and allowed to stand at room temperature, when a yellow solid separated out. Crystallisation from ethanol gave pale yellow crystals melting at 215°.

Analysis: Found: C, 59.86; H, 3.30

Calculated for $C_{13}H_8O_6$: C, 60.01; H, 3.10%.

Acetone:

The heraclenin (1 g.) dissolved in glacial acetic acid (15 cc), chromium trioxide (0.4 g.) dissolved in 50% aqueous acetic acid (20 cc) was added and reaction allowed to stand at room temperature for 24 hours. It was neutralised with a solution of sodium hydroxide with constant cooling and immediately steam distilled. The distillate was directly collected in an aqueous solution of 2:4-dinitrophenylhydrazine sulphate when an orange-red precipitate of acetone 2:4-dinitrophenylhydrazone was obtained. It was crystallised from acetone free methanol melting point 124-125°. No depression in melting point was observed when admixed with an authentic sample.

Partial synthesis of heraclenin:

Imperatorin (800 mg.) was dissolved in chloroform (5 cc) and a solution of perbenzoic acid (500 mg.) in chloroform was added with constant shaking. The reaction mixture was allowed to stand at room temperature for three days, diluted with ether. The ether-chloroform layer was washed with 5% aqueous solution of sodium bicarbonate and then with water and dried over anhydrous sodium sulphate The solvent was filtered off and concentrated to dryness. The residue was dissolved in benzene and chromatographed

over acetic acid deactivated alumina. The benzene eluate on evaporation gave thick viscous product which on crystallisation from benzene-petroleum ether mixture melted at 114-115°, identical with that reported by Späth.

Analysis: Found: C, 67.19; H, 5.07.

Calculated for $C_{16}H_{14}O_5$: C, 67.12; H, 4.93%.

8-Geranoxypsoralen:

8-Geranoxypsoralen melting point 53-54°, λ max. 215 (log ε 4.51), 248 (log ε 4.42) and 298 mμ (log ε 4.13) which was obtained from the petroleum ether eluate of the column gave the following analytical data:

Analysis: Found: C, 74.32; H, 6.73.

Calculated for $C_{21}H_{24}O_4$, C, 74.09; H, 7.11%.

Xanthotoxol:

8-Geranoxypsoralen (100 mg.) was dissolved in glacial acetic acid (2 cc) and after adding concentrated sulphuric acid (2 drops) the mixture was heated on a water bath for 20 minutes. The reaction mixture was cooled to room temperature and then poured over crushed ice in a beaker. The separated solid was sublimed at 190-200° (bath)/ 1 mm. The sublimate was crystallised from ether melting point 248-249°.

Analysis: Found: C, 65.08; H, 3.14

Calculated for $C_{11}H_6O_4$: C, 65.35; H, 2.99%.

Xanthoxtoxol acetate:

Xanthotoxol (100 mg.) acetic anhydride (2 cc) and fused sodium acetate (20 mg.) were refluxed on a sand bath for 1 hour and then worked up as described earlier.

Xanthotoxin:

Xanthotoxol (100 mg.) dissolved in methanol (4 cc) was treated with an excess of ethereal solution of diazomethane and the reaction mixture allowed to stand

overnight and then worked up in the usual manner.

Xanthotoxin nitrate:

Xanthotoxin (100 mg.) in glacial acetic acid (1 cc) was treated with concentrated nitric acid (sp. gr. 1.42, 5 drops) at room temperature and then worked up as described earlier.

Geranyl acetate:

8-Geranoxypsoralen (1 g.) and glacial acetic acid (1 cc were heated together in an oil bath at 115-120° for 1½ hour and the reaction mixture was allowed to stand at room temperature overnight. It was extracted five times with n-hexane and the combined hexane extracts were washed with 5% aqueous solution of sodium bicarbonate and then with water and dried over anhydrous sodium sulphate. The solvent was filtered off and the filtrate evaporated to dryness. The residue thus obtained was distilled in a micro distilling apparatus under vacuum. The pleasant smelling oil thus obtained was identified as geranyl acetate by superimposable IR spectrum of an authentic sample as well as by vapour phase chromatography.

Geranyl alcohol:

Geranyl acetate (200 mg.) was refluxed with 10% methanolic potassium hydroxide (10 cc) for 2 hours on a water bath. The solvent was removed under reduced pressure and the residue diluted with water. The aqueous extract was extracted with hexane and the hexane layer was washed

with water and dried over anhydrous sodium sulphate. The solvent was filtered off and concentrated to dryness. The residual oil was then distilled in a microdistilling apparatus at 90-95°/3 mm. The sweet smelling oil obtained analysed for the following values.

Analysis: Found: C, 78.06; H, 11.92

Calculated for $C_{10}H_{18}O$: C, 77.86; H, 11.76%.

It was characterised as geranyl alcohol by superimposable IR spectrum of an authentic sample and vapour phase chromatography.

Geranyl-3,5-dinitrobenzoate:

To geraniol (100 mg.) dissolved in dry benzene was added a solution of 3,5-dinitrobenzoyl chloride (200 mg) in dry benzene (10 cc). To the mixture a few drops of dry pyridine were added and it was refluxed on a water bath for ½ hour. The reaction mixture was cooled and diluted with an excess of ether. The ether-benzene layer was shaken first with 5% aqueous solution of sodium bicarbonate and then with water, dried over anhydrous sodium sulphate and filtered. The solvent was removed under reduced pressure and the residue was kept in a refrigerator when a white solid separated out. The product was washed with cold petroleum ether to remove any unreacted oil, the solid thus remained was crystallised from methanol melting point 59-60°. No depression was observed when admixed with an authentic sample of geranyl-3,5-dinitrobenzoate.

Ozonolysis of 8-geranoxypsoralen:

Ozonised oxygen was passed into a solution of 8-geranoxypsoralen (1 g.) in glacial acetic acid (40 cc) untill oxidation was complete (2 hours). Water (50 cc) and zinc dust (500 mg.) were added and the reaction mixture warmed on a water bath till clear. More water (100 cc) was then added and the solution was steam distilled, the distillate being collected in fractions directly into an aqueous solution of 2,4-dinitrophenyl-hydrazine sulphate gave bulky orange precipitate which dissolved almost completely in hot acetone free methanol. The filtered solution, on cooling, deposited orange needles of acetone-2,4-dinitrophenylhydrazone melting point 124-125°. No depression in melting point on admixture with an authentic sample.

Later fractions of steam distillate gave a bright yellow precipitate, insoluble in hot alcohol. This was crystallised from nitrobenzene-alcohol mixture melting point 232-234°, identified as laevulinic aldehyde.

Birch reduction of Compound B:

A three necked 500 ml. round bottom flask was fitted with a dry ice condenser, stirrer and a dropping funnel. Ammonia (75 cc) was condensed in the flask and sodium metal (3 g.) was added in small pieces, producing a blue solution. To the vigorously stirring Na/NH_3 solution

under nitrogen atmosphere compound B (500 mg.) in 20 cc of methyl alcohol was added dropwise over half an hour. The blue colour of Na/NH_3 disappeared during the addition, some more sodium was added in small pieces to maintain the blue colour for 1 hour. Then heptane (25 cc), granulated NH_4Cl (10 g.) and water (50 cc) were added to the refluxing reaction mixtures. After separating the heptane layer, the aqueous layer was washed with three 25 cc portions of heptane. The combined heptane extracts were washed with water, untill the washings were neutral, dried over anhydrous magnesium sulphate and subjected to VPC analysis. The VPC analysis showed peaks attributed to 2-methyl-2-butene and trans-2,6-dimethyl-2,6-octadiene (methylgeraniolene).

Synthesis of 8-geranoxypsoralen:

Geranyl bromide:

Phosphorus tribromide (12 gm.) in petroleum ether (40-60°) (10 ml.) was added dropwise during one hour at -7° to geraniol (15 g.) in petroleum ether 29 ml. and pyridine (2.5 ml.) with constant stirring. The reaction mixture was further stirred for 20 minutes at -7°, poured over crushed ice, stirred for 15 minutes and then treated with petroleum ether (100 ml.). The petroleum ether layer was separated, washed twice with water and once with dilute solution of sodium bicarbonate, dried (Na_2SO_4) and solvent evaporated off. The residue was distilled at 102-103° at 3 mm. pressure.

Condensation of xanthotoxol and geranyl bromide:

A mixture of xanthotoxol (0.2 g.), anhydrous potassium carbonate (3 g.), anhydrous acetone (50 ml.) and geranyl bromide (2 ml.) was refluxed for 36 hours. Acetone solution was filtered and the residue washed five times with 20 ml. portions of dry acetone. The acetone washings were combined with the main bulk and solvent removed. The residue dissolved in a minimum amount of dry benzene and chromatographed over acetic acid deactivated alumina, elution with petroleum ether gave a viscous mass, which crystallised from petroleum ether in needles melting point and mixed melting point 53-54°. The IR spectra of natural and synthetic products were superimposable.

Benzene extract:

The petroleum ether exhausted Heracleum candicans root powder (5 kg.) was extracted with benzene in a soxhlet for 80 hours. The solvent was removed under reduced pressure and the residual viscous mass (500 g.) was purified by column chromatography over silica gel. Silica gel (100 g.) was made into a slurry with dry n-hexane the slurry then poured into a column with constant tapping. The above viscous mass (10 g.) was dissolved in the minimum amount of ethyl acetate and was mixed well with silica gel (25 g.). To this mixture hexane was added and the slurry was poured over the top of the column. The column was then eluted with hexane with gradually increasing the percentage of ethyl acetate. The hexane-ethyl acetate (1:1) eluate, on concentrating, gave a thick mass (500 mg.) which could be crystallised from hexane-ethyl acetate mixture. On further crystallisations from ethyl acetate alone it melted at 117-118°.

Heraclenol:

Heraclenol melting point 117-118° $(\alpha)_D^{32} = +16.5$ (pyridine) λ max. 249 (log ϵ 4.24), 305 (log ϵ 4.0) and 320 mμ (log ϵ 3.88), analysed for the following values.

Analysis: Found: C, 63.25; H, 5.35.

Calculated for $C_{16}H_{16}O_6$: C, 63.15; H, 5.30%.

Xanthotoxol:

Heraclenol (200 mg.) was dissolved in glacial acetic acid (4 cc) to which concentrated sulphuric acid (4 drops) was added. The reaction mixture was heated on boiling water bath for 45 minutes, allowed to stand at room temperature overnight, and then poured over crushed ice in a beaker. The gummy mass thus separated was filtered off, dried and sublimed at 190-200° (bath)/1 mm. The sublimate was crystallised from ether melting point 248-249°.
Analysis: Found: C, 64.99; H, 3.16.

Calculated for $C_{11}H_6O_4$: C, 65.35; H, 2.99%

Xanthotoxol acetate:

Xanthotoxol (100 mg.) acetic anhydride (2 cc) and fused sodium acetate (20 mg.) were refluxed on a sand bath for 1 hour and then worked up as described earlier.

Xanthotoxin:

Xanthoxol (100 mg.) was dissolved in methanol (4 cc), treated with an excess of ethereal solution of diazomethane and the reaction mixture allowed to stand overnight and then worked up in the usual manner.

Xanthotoxin nitrate:

Xanthotoxin (100 mg.) in glacial acetic acid (1 cc) was treated with concentrated nitric acid (sp.gr. 1.42 5 drops) at room temperature and then worked up as described earlier.

Isoheraclenin:

Heraclenol (500 mg.) was dissolved in dry toluene (25 cc) and the solution was brought to boiling. Phosphorou pentoxide (2 g.) was then added to the boiling mixture and boiling continued for further 10 minutes. The reaction mixture was cooled, filtered and the filtrate was diluted with an excess of ether. The ether-toluene layer was washed successively with water, 5 per cent aqueous sodium bicarbonate and finally with water, dried over anhydrous sodium sulphate. The solvent was filtered off and concentrated to dryness under reduced pressure. The residue was crystallised from ether melting point 132-34°. In a mixed melting point determination with an authentic sample of isoheraclenin, no depression in melting point was observed.

Analysis: Found: C, 67.23; H, 5.10

Calculated for $C_{16}H_{14}O_5$: C, 67.12; H, 4.93%.

Chromic acid oxidation:

To heraclenol (1 g.) dissolved in glacial acetic acid (15 cc) was added chromium trioxide (0.4 g.) dissolved in 50% aqueous acetic acid (20 cc). The reaction mixture was allowed to stand at room temperature overnight. It was diluted with water and immediately extracted with a large excess of ether. The ethereal layer was washed with water and dried over anhydrous sodium sulphate.

The solvent was filtered off, and concentrated to dryness under reduced pressure. The reddish brown residue left behind was dissolved in minimum amount of methyl alcohol and treated with diazomethane in ether. The reaction mixture was allowed to stand in a refrigerator overnight. The solvent was removed and the residual methyl ester was dissolved in dry benzene and chromatographed over acetic acid deactivated alumina. The benzene eluate on evaporation gave a thick mass which on crystallisation from methanol melted at 145-147°.

Analysis: Found: C, 61.10; H, 3.78

Calculated for $C_{14}H_{10}O_6$: C, 61.32; H, 3.68%.

The above methyl ester (300 mg.) was refluxed with 50% aqueous acetic acid (16 cc) for 1 hour. The reaction mixture was cooled and then diluted with water to 50 cc. On standing for a few hours a yellow crystalline mass separated out. It was filtered dried and recrystallised from ethanol to give pale yellow needles melting point 215°.

Analysis: Found: C, 59.72; H, 3.30

Calculated for $C_{13}H_8O_5$: C, 60.01; H, 3.10%.

In another similar experiment, the reaction mixture was neutralised with an aqueous solution of sodium hydroxide with constant cooling and immediately steam distilled. The steam distillate was directly collected in an aqueous solution of 2,4-dinitrophenylhydrazine sulphate when a bulky orange precipitate was obtained.

It was dried and crystallised from acetone free methanol in orange red needles melting point 124-125°. Identified as acetone-2,4-dinitrophenylhydrazone by mixed melting point with an authentic sample.

Seseli sibiricum:

Extraction:

The powdered air dried Seseli sibiricum roots (10 kg.) were refluxed with petroleum ether (80-100°) 20 litres on a water bath for 24 hours. The extract was filtered while hot and the process was repeated three times. The combined extracts on concentration under reduced pressure gave an oily mass (800 g.).

Chromatography of the oily mass:

Deactivated alumina (400 g.) was made into a slurry with dry, petroleum-ether (40-60°) and the slurry poured into a column. The oily mass (20 g.) dissolved in minimum amount of benzene was run through the column, it was eluted with petroleum ether (40-60°). The petroleum ether eluate on concentration gave an oily mass which on keeping in an ice chest for 72 hours deposited long needles (1.25 g.) identified as osthol. Further elution of the column with benzene removal of the solvent from the eluate and crystallisation of the residue gave a crystalline mass (250 mg.) characterised as fenchyl p-hydroxy cinnamate.

Osthol:

Osthol melting point 84-85°, λ max. 215 (log ε 4.25) 251 (log ε 3.65), 258 (log ε 3.68) and 322 mμ (log ε 4.19), obtained from the petroleum ether eluate of the column analysed for the following values:

Analysis: Found: C, 73.99; H, 6.74.

Calculated for $C_{15}H_{16}O_3$: C, 73.75; H, 6.6%.

Ostholic acid:

To osthol (1 g.) dissolved in glacial acetic acid (15 cc) chromium trioxide (800 mg.) dissolved in 50 % aqueous acetic acid (20 cc) was added and the reaction mixture allowed to stand at room temperature for 3 days. The reaction mixture was diluted with water and extracted immediately with a large excess of ether. The ethereal layer was washed with water, dried over anhydrous sodium sulphate and solvent was removed under reduced pressure. The residue was dissolved in water and allowed to stand for some time when light yellow crystals of ostholic acid separated out, melting point 253-254°.

Ostholic acid methyl ester:

Ostholic acid (500 mg.) dissolved in methanol (10 cc) was treated with diazomethane in ether. The reaction mixture was allowed to stand overnight, and the solvent was removed. The residue was crystallised from methanol melting point 140-141°.

7-Methoxy-8-methyl coumarin:

To Ostholic acid (1.5 g.) in quinoline (80 cc) were added copper turnings (8 g.) and the reaction mixture was refluxed for 45 minutes on a sand bath. It was cooled, diluted with an excess of ether and filtered. The filtrate was washed several times with 3% aqueous hydrochloric acid and then with water and dried over sodium chloride. The solvent was removed and the dark coloured residue was dissolved in benzene, and chromatographed over acetic acid deactivated alumina. Elution of the column with benzene and evaporation of the solvent gave a slightly coloured mass, crystallised from benzene-petroleum ether mixture melting point 136-137^{o} (Sublime).

Fenchyl p-hydroxy cinnamate:

Fenchyl p-hydroxy cinnamate melting point 215-216°, λ max. 230 (log ϵ 4.19) and 320 mμ(log ϵ 4.45) gave the following analytical values.

Analysis: Found: C, 75.80; H, 8.36.

Calculated for $C_{19}H_{24}O_3$: C, 75.97; H, 8.06%.

Acetate of fenchyl p-hydroxy cinnamate:

Fenchyl p-hydroxy cinnamate (500 mg.) acetic anhydride (5 cc) and pyridine (0.5 cc) were heated together on a water bath for two hours and then kept at room temperature overnight. The reaction mixture was poured over crushed ice contained in a beaker, allowed to stand with occasional stirring when a colourless substance separated out, The solid product was filtered off and crystallised from methanol in colourless needles, melting point 95°.

Analysis: Found: C, 73.35; H, 7.51

$C_{21}H_{26}O_4$ requires C, 73.66; H, 7.66%.

Hydrolysis of fenchyl p-hydroxy cinnamate:

Isolation of Fenchyl alcohol:

Fenchyl p-hydroxy cinnamate (1 g.) and methanolic potassium hydroxide 10% (50 cc) were refluxed for 4 hours on a water bath. The solvent was removed under reduced pressure and the residue diluted with water (10 cc) and extracted thrice with 50 cc portions of ether. The ethereal layer was washed with water, dried over anhydrous sodium sulphate and the solvent filtered off. Evaporation of the solvent gave an oily residue which was distilled at

70°/16 mm. The distillate on keeping in an ice chest solidified to a colourless crystalline mass melting point 39-40°.

Isolation of p-coumaric acid:

The aqueous layer from the above alkaline hydrolysate was acidified with dilute hydrochloric acid and then extracted with ether. The ethereal extract was washed with water, dried over anhydrous sodium sulphate and the solvent removed. The residue was crystallised from petroleum ether (40-60°) to colourless needles melting point 208-212°. This was further purified by sublimation in vacuum. The sublimate melts at 212-214°.

Analysis: Found: C, 65.92; H, 5.24

Calculated for $C_9H_8O_3$: C, 65.85; H, 4.91%.

Acetate of p-coumaric acid:

p-Coumaric acid (100 mg.) acetic anhydride (2 cc) and pyridine (0.2 cc) were heated together on a water bath for 2 hours and then left at room temperature overnight. The reaction mixture was poured over crushed ice and stirred well. The separated colourless mass was filtered off and crystallised from methanol melting point 208-210°.

Hydrolysis of fenchyl p-hydroxy cinnamate mono-methyl ether:

Fenchyl p-hydroxy cinnamate (1 g.) dissolved in methyl alcohol (5 cc) was treated with diazomethane in ether and left overnight. The solvent was removed and the residue left behind failed to crystallise. This crude

product was then hydrolysed with 10% methanolic potassium hydroxide (25 cc) by refluxing for 4 hours on a water bath. The solvent was removed under reduced pressure and the residue was diluted with water and extracted with ether. The ethereal solution was washed with water, dried over anhydrous sodium sulphate and the solvent was filtered off. Evaporation of the solvent and distillation of the residue gave fenchyl alcohol.

The aqueous layer from alkaline hydrolysate was acidified with dilute hydrochloric acid and extracted with ether. The ethereal layer was washed with water and dried over anhydrous sodium sulphate, the solvent was filtered and evaporated off. The residue was crystallised from methanol when colourless needles of p-methoxy cinnamic acid were obtained, melting point 175°.

p-Nitrobenzoate of fenchyl alcohol:

Fenchyl alcohol (100 mg.) and p-nitrobenzoyl chloride (100 mg.) were heated on a water bath for 45 minutes. The mixture was cooled and the product was decomposed with 5 per cent aqueous solution of sodium bicarbonate. The solid thus obtained was filtered on a Hirsh funnel and washed first with 5% aqueous sodium bicarbonate solution and then with water. On crystallisation from methanol slightly coloured rods of p-nitro benzoate of fenchyl alcohol were obtained, melting point 107-109°.

Analysis: Found: C, 67.27; H, 6.68.

Calculated for $C_{17}H_{21}O_4N$: C, 67.31; H, 6.98%.

Synthesis of fenchyl p-hydroxy cinnamate:

p-Acetoxy cinnamoyl chloride:

p-Acetoxy cinnamic acid (500 mg.) and thionyl chloride (1 cc) were heated together on a water bath for 45 minutes. The excess of thionyl chloride was distilled off on the water bath under reduced pressure. The crude product left behind was used immediately as such.

Condensation of p-acetoxy cinnamoyl chloride and fenchyl alcohol:

p-Acetoxy cinnamoyl chloride (500 mg.) and fenchyl alcohol (1 cc) were heated together on a water bath for 45 minutes. The reaction mixture was cooled and titurated with 5% aqueous solution of sodium bicarbonate and then washed with water on a Hirsh funnel till free from alkali. The gummy mass left behind was crystallised from methanol, melting point 216-217°. In a mixed melting point determination with an authentic sample no depression in melting point was observed.

Angelica glauca Edgw:

Extraction:

Powdered air dried seeds of Angelica glauca (300 g.) were extracted with hexane in a soxhlet apparatus for 36 hours. The extract on concentration and cooling depsited a yellow solid (3 g.) which was filtered and the filtrate again concentrated and kept in refrigerator when a further quantity (3 g.) of the solid separated out. Total yield amounted to 2 per cent.

The solid was dissolved in ether and extracted thrice with 5% aqueous sodium bicarbonate and once with water. The organic phase was dried over anhydrous sodium sulphate, filtered and solvent removed. Crystallisation from ether-hexane afforded a yellow crystalline mass.

Thin layer chromatographic analysis on alumina plates run with a mixture of ethyl acetate-hexane showed it to be a mixture of three components.

Separation of the components:

The above mixture (2 g.) was made into a slurry with ethyl acetate and alumina (25 g.) and the solvent completely evaporated to give a homogeneous deposit of the material over alumina. The alumina was placed on a column of deactivated alumina (125 g.) and eluted with hexane-ethyl acetate mixture while gradually increasing the amount of ethyl acetate. 100 cc fractions of the eluate were collected and each fraction was checked for its homogeneity on chromatostrips.

Fraction Nos.	Compound	Melting point
1-8	Single 'A'	109-110°
9-41	Mixture	
42-60	Single 'B'	103-104°
61-65	Mixture	
66-75	Single 'C'	119-120°

The mixed fractions were collected and rechromatographed.

Isoimperatorin:

Fraction 'A' melting point 109-110° (methanol) had the following peaks in its ultraviolet spectrum $\lambda_{max.}^{methanol}$ 222,250,268 and 310 mμ.

Analysis: Found: C, 70.54; H, 5.17

Calculated for $C_{16}H_{14}O_4$: C, 71.10; H, 5.22%.

Bergaptol:

Isoimperatorin (100 mg.) was dissolved in glacial acetic acid (2 cc) containing concentrated sulphuric acid (2 drops) and heated on a water bath for 45 minutes. The reaction mixture was left overnight at room temperature, poured over crushed ice and the separated material washed thoroughly with water, melting point 278-280°. No depression in mixed melting point with an authentic sample.

Bergapten:

Bergaptol (200 mg.) was methylated with ethereal solution of diazomethane. Removal of the solvent gave brownish colour residue which was dissolved in benzene and chromatographed over deactivated alumina. Benzene eluate gave colourless needles melting at 189°. No depression was observed in a mixed melting point determination with an authentic sample of bergapten.

Prangolarin:

Fraction 'B' was crystallised from hexane-ethyl acetate mixture and melted at 103-104°, and showed a molecular ion peak at 286 in its mass spectrum. Ultraviolet spectrum showed absorptions at $\lambda_{max.}^{methanol}$ 222, 250, 268 and 310 mμ.

Analysis: Found: C, 66.75; H, 5.14

Calculated for $C_{16}H_{14}O_5$: C, 67.12; H, 4.93%.

Bergaptol:

Prangolarin (100 mg.) glacial acetic acid (2 cc) and concentrated sulphuric acid (2 drops) were heated on a water bath for 45 minutes and worked up as described earlier. The solid obtained was identified as bergaptol.

Compound C:

The third component of the mixture, fraction C, after crystallisation from ethyl acetate melted at 119-120°, it showed a molecular ion peak at 318 in its mass spectrum. The ultraviolet spectrum showed absorptions at, $\lambda_{max.}^{methanol}$ 220, 250, 268 and 310 mμ.

Analysis: Found: C, 63.96; H, 5.69; OMe, 9.3

$C_{17}H_{18}O_6$ requires: C, 64.14; H, 5.70; OMe, 9.7%.

B I B L I O G R A P H Y

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CHEMICAL EXAMINATION OF *HERACLEUM CANDICANS*—I

ISOLATION AND STRUCTURE OF A NEW FUROCOUMARIN—HERACLENIN

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Abstract—A new furocoumarin, heraclenin, has been isolated from *Heracleum candicans* and its structure has been established as 8-(β,γ-oxido-isoamyloxy)-psoralen.

SKIN photosensitizing action of a number of furocoumarins has been discussed by Musajo and Rodighiero[1] and Pathak and Fitzpatrick.[2-3] The results of these investigations have established that the essential requirement for this property is the presence of a linear furocoumarin nucleus. Furocoumarins having free phenolic hydroxy groups are inactive but the activity is restored on alkylation. As regards these alkyl ethers, the methyl and ethyl ethers are most active and from there on the activity is gradually reduced with the lengthening of the side chain, even so oxypeucedanin was found to possess photosensitizing activity.

In this connexion it was felt that investigation of other naturally occurring coumarins could be of some interest. We report here the isolation of a new furocoumarin, heraclenin(I) which has been obtained from *Heracleum candicans* (Umbelliferae).

Heraclenin (I) m.p. 111°, $(\alpha)_D^{32} = +22$ analyses for $C_{16}H_{14}O_5$ and its UV spectrum is similar to that of imperatorin(II) (Fig. 1). The molecular formula differs from that of imperatorin by the presence of an extra oxygen atom. Treatment of the compound with acetic acid–sulphuric acid mixture, under conditions generally used for the cleavage of the side chain in furocoumarins, affords a phenolic product (III) $C_{11}H_6O_4$ m.p. 248–249°, which gives a pale green ferric chloride reaction, forms an acetate m.p. 178° and methyl ether m.p. 146–147°. These agree with the m.p. of xanthotoxol (III) and xanthotoxin (IV) respectively. Xanthotoxol acetate has not been reported. Identity of the cleavage product with xanthotoxol was confirmed by comparison with an authentic sample.

The extra oxygen atom in heraclenin must, therefore, be present in the C_5H_9 side chain, possibly in an epoxide linkage since the IR spectrum of the compound does not show hydroxy band. This is supported by the hydrolysis, under mild acidic conditions, to a diol (V) $C_{16}H_{16}O_6$ m.p. 117–118°. Oxidation with chromic acid in acetic acid gave acetone and an acid (VI) $C_{13}H_8O_6$ m.p. 215°. This indicates that the C_5H_9O residue in heraclenin is similarly constituted as in the isomeric oxypeucedanin

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[2] M. A. Pathak and T. R. Fitzpatrick, *J. Investig. Dermatol.* **32,** 255 (1959).
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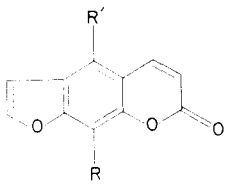

I R = O—CH_2—CH(O)—C(CH_3)(CH_3) R′ = H

II R = O—CH_2—CH=C(CH_3)(CH_3) R′ = H

III R = OH R′ = H

IV R = OCH_3 R′ = H

V R = O—CH_2—CH(OH)—C(OH)(CH_3)(CH_3) R′ = H

VI R = O—CH_2—COOH R′ = H

VII R = H R′ = O—CH_2—CH(O)—C(CH_3)(CH_3)

VIII R = H R′ = O—CH_2—COOH

IX R = O—CH_2—C(=O)—CH(CH_3)(CH_3) R′ = H

(VII), which gives acetone and oxypeucedaninic acid (5-ω-carboxymethoxy-4′,5′,6,7-furocoumarin), on chromic acid oxidation. Compound VI has accordingly been formulated as 8-ω-carbomethoxy-4′,5′,6,7-furocoumarin. Schönberg and Sina,[4] who synthesized this acid, have reported 210° as the melting point.

On refluxing in toluene over phosphorous pentoxide or on boiling with dilute mineral acids heraclenin is converted in good yield to a ketone (IX) $C_{16}H_{14}O_5$ m.p. 132–134° formed by opening and rearrangement of the epoxide ring. The IR spectrum of this compound has a doublet in the carbonyl region (5·75 and 5·8 μ). It has been named isoheralcenin in conformity with the nomenclature adopted by Späth for the rearrangement product of oxypeucedanin.[5] Treatment of oxypeucedanin with sodium acetate–acetic anhydride gives a diacetate, however, heraclenin does not form a diacetate on refluxing with this mixture. In all other reactions heraclenin and oxypeucedanin are analogous.

The structure of heraclenin (I) as 8-(β,γ-oxido-isoamyloxy)-psoralen was further confirmed by the oxidation of imperatorin with perbenzoic acid according to Späth,[6]

[4] A. Schönberg and A. Sina, *J. Amer. Chem. Soc.* **72,** 4826 (1950).
[5] E. Späth and K. Klager, *Ber. Dtsch. Chem. Ges.* **66,** 914 (1933).
[6] E. Späth and H. Holzen. *Ber. Dtsch. Chem. Ges.* **68,** 1123 (1935).

when a compound m.p. 114–115°, reported by him, was obtained. The synthetic compound oxyimperatorin, being racemic, gives a depression in m.p. with heraclenin (I) but has a superimposable IR spectrum.

Heraclenin has not so far been reported to occur naturally though its presence in the extract of masterwort (*Imperatoria ostruthium*) was suspected by Späth.[6]

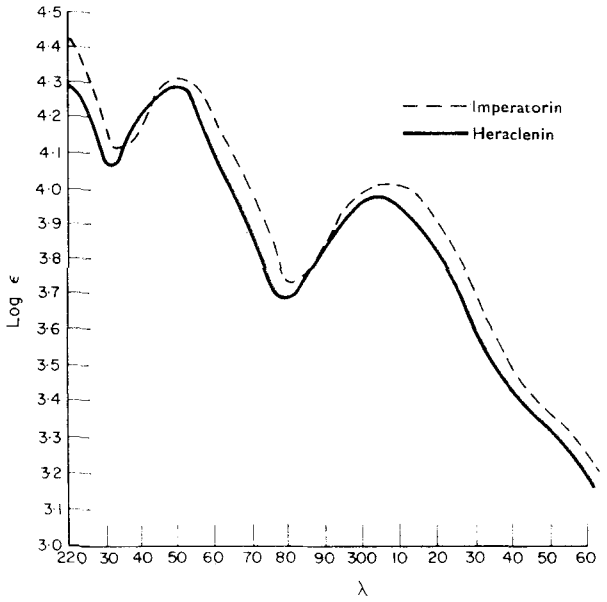

FIG 1

EXPERIMENTAL

All UV spectra were measured in a Beckmann model DU instrument in 95% ethanol. IR spectra were taken on a Perkin Elmer Infracord either in chloroform solutions or as mulls in nujol.

Isolation of heraclenin (I). Air dried finely powdered roots (2 kg) of *Heracleum candicans* were extracted with pet. ether (40–60°) in a soxhlet for 20 hr. On cooling the extract deposited a yellow solid (120 g), 5 g of which was dissolved in benzene and chromatographed over deactivated alumina (250 g) prepared by shaking with 10% aqueous acetic acid for 2 hr. Elution with pet. ether (40–60°) afforded a small quantity of a low melting product. Further elution with benzene–pet ether mixture (1:5) gave a crystalline product m.p. 82°. Finally, elution with benzene–pet ether mixture (1:1) yielded heraclenin (1·5 g). After crystallization from methanol it melted at 111°, $(\alpha)_D^{32} = +22$ (pyridine), λ_{max} 250 mμ (log ϵ 4·31), 305 mμ (log ϵ 4·02). (Found: C, 67·08; H, 4·99; $C_{16}H_{14}O_5$ requires: C, 67·12; H, 4·93%).

Xanthotoxol (III). Heraclenin (1 g) was dissolved in glacial acetic acid (20 ml) to which conc H_2SO_4 (20 drops) were added. The reaction mixture was heated on a water bath for 30 min, cooled and then poured over crushed ice. The gummy substance which separated out was sublimed at 1 mm, 190–200° (bath temp). Crystallization of the sublimate from ether gave crystals of xanthotoxol m.p. 248–249°.

Xanthotoxin (IV). Xanthotoxol (200 mg) in methanol (2 ml) was treated with excess diazomethane in ether and left overnight. The residue was dissolved in benzene and chromatographed over deactivated alumina with the same solvent. Xanthotoxin was crystallized from benzene–pet ether m.p. 146–147°.

Xanthotoxol acetate. Xanthotoxol (500 mg) acetic anhydride (5 ml) and fused sodium acetate (100 mg) were refluxed 1 hr. The mixture was cooled, poured over crushed ice and the acetate was crystallized from methanol m.p. 178°. (Found: C, 64·88; H, 3·51; $C_{13}H_8O_5$ requires: C, 63·94; H, 3·30%).

Heraclenin hydrate (V). A solution of heraclenin (200 mg) in water (50 ml) was heated on a water bath, oxalic acid (50 mg) was added and heating continued for 10 min. The material which separated on cooling was washed with water and crystallized from ethyl acetate m.p. 117–118°. (Found: C, 63·28; H, 5·39, $C_{16}H_{16}O_6$ requires: C, 63·15; H, 5·30%).

Isoheraclenin (IX). Heraclenin (1 g) was dissolved in dry toluene (50 ml) and brought to boiling P_2O_5 (4 g) was added and the mixture refluxed for another 10 min, cooled and filtered. The filtrate was diluted with ether and the ether–toluene solution extracted with $NaHCO_3$aq., washed with water and dried (Na_2SO_4). The solvent was removed under vacuum and the residue crystallized from ether, m.p. 132–134°. It formed a crystalline 2,4-dinitrophenylhydrazone. (Found: C, 67·33; H, 5·06; $C_{16}H_{14}O_5$ requires: C, 67·12; H, 4·93%). Heraclenin was also isomerized to isoheraclenin with 10% H_2SO_4.

8-*ω-Carbooxymethoxy*-4′,5′,6,7-*furocoumarin* (VI). To heraclenin (3 g) dissolved in glacial acetic acid (45 ml), chromium trioxide (1·2 g) in 50% aqueous acetic acid (60 ml) was added, and the solution allowed to stand for 24 hr at room temp. The reaction mixture was diluted with water and extracted with a large excess of ether. The ethereal solution was washed with water dried (Na_2SO_4) and solvent removed under vacuum. The reddish brown residue obtained was dissolved in methyl alcohol and methylated with excess diazomathane in ether. The methylated product was dissolved in benzene and chromatographed over acid treated alumina. The benzene eluate on evaporation deposited pale yellow needles. It was crystallized from alcohol m.p. 146–147° (Found: 61·16; H, 3·73; $C_{14}H_{10}O_6$ requires: C, 61·32; H, 3·68%).

The above ester (0·3 g) was refluxed with 50% acetic acid (16 ml) for 1 hr. It was cooled and diluted with water to 50 ml and after standing a yellow coloured solid separated. This crystallized from alcohol in pale yellow crystals m.p. 215° (m.p. reported by Schönberg and Sina 210°).[4] (Found: C, 59·66; H, 3·30; Calc. for $C_{13}H_8O_6$: C, 60·01; H, 3·10%).

Acetone. In another experiment the reaction mixture obtained by the above oxidation was neutralized with NaOH under cooling and immediately steam distilled. The distillate was collected in an aqueous solution of 2:4-dinitrophenylhydrazine and the 2,4-dinitrophenylhydrazone derivative of acetone crystallized from methanol and was identified by mixed m.p. with an authentic sample.

Oxy-imperatorin. Imperatorin (0·003 moles) was dissolved in chloroform (2 ml) and a solution of perbenzoic acid (0·004 moles) in chloroform was added with gradual shaking. The reaction mixture was then allowed to stand at room temp for 3 days, diluted with ether and washed with $NaHCO_3$aq. The ether–chloroform layer was dried (Na_2SO_4). The solvent was removed and the residue dissolved in benzene and chromatographed over deactivated alumina. The oily substance obtained crystallized from benzene–pet ether (40–60°) m.p. 114–115°, and was identical with that reported by Späth. (Found: C, 67·19; H, 5·07; Calc. for $C_{16}H_{14}O_5$: 67·12; H, 4·93%).

This product was found to be identical with heraclenin by superimposable IR spectrum.

Acknowledgements—The authors wish to thank Dr. M. L. Dhar, Director, Central Drug Research Institute, Lucknow, for a sample of imperatorin, Dr. M. M. Dhar for infrared spectra, Mr. V. P. Mahajan for optical rotation and Ministry of Health Government of India, for financial assistance.

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Chemical Examination of Heracleum candicans. II

Isolation and Structure of a new Furocoumarin, Heraclenol

Von
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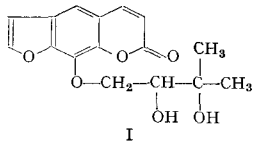
Chemical Examination of Heracleum candicans. II
Isolation and Structure of a New Furocoumarin, Heraclenol

Isolation and structure of a new furocoumarin, heraclenin, from petroleum ether extract of the roots of Heracleum candicans (Umbelliferae) has already been reported in a previous communication. After extraction with petroleum ether the drug was further subjected to extraction with hot benzene in a soxhlet, and another furocoumarin, heraclenol (I), $C_{16}H_{16}O_6$, m.p. 117—118°, $(\alpha)_D^{32} = +16.5$ (pyridine) was obtained. It was purified by column chromatography over silicic acid with n-hexane and ethyl acetate mixture (1 : 1) and crystallized first from ethyl acetate-n-hexane and then from ethyl acetate alone.



I

The ultraviolet spectrum of the compound was similar to that of heraclenin and the infrared spectrum also indicated presence of a furocoumarin nucleus. Degradation of this by acetic acid-sulphuric acid mixture gave a phenolic product, $C_{11}H_6O_4$, m.p. 248—49°, identified as xanthotoxol by comparison with an authentic sample. Chromic acid oxidation afforded an acid, $C_{13}H_8O_6$, m.p. 215°, identified as 8-ω-carboxymethoxy-4′,5′,6,7-furocoumarin identical with the degradation product obtained from heraclenin.

Heraclenol when subjected to dehydration with P_2O_5 in toluene gave a ketone, $C_{16}H_{14}O_5$, m.p. 132—134° identical with isoheraclenin (m.m.p.).

Identity of heraclenol as 8-(β,γ-dihydroxyisoamyloxy)-psoralen (I)[1]) was finally established by comparison with an authentic sample, which was earlier synthesized by us through the oxalic acid hydrolysis of heraclenin and named heraclenin hydrate. Both the samples gave no depression in a mixed m.p. determination and had superimposable IR spectra.

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[1]) Sharma, Y.N., A. Zaman, and A.R. Kidwai: Tetrahedron **20**, 87 (1964).

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COUMARIN CONSTITUENTS OF HERACLEUM CANDICANS-III

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Abstract:

8-Geranoxypsoralen was isolated from H. candicans and characterised by degradations and synthesis.

In previous communications the isolation of two furocoumarins heraclenin[1] and heraclenol[2], from Heracleum candicans roots was reported. The former compound was obtained when the crude coumarin mixture from the peteroleum ether extract of the plant roots was chromatographed over deactivated alumina[3] using petroleum ether-benzene in the ratio of 1:1 for elution, and was found to be the major coumarin constituent of the plant. Evaporation of the petroleum ether and petroleum ether-benzene (5:1) eluates also gave two other compounds in small amounts which crystallised well and had melting points (A) 53-54° and (B) 82-83° respectively. Heraclenol mentioned above is not present in the petroleum ether extract and was obtained on subsequent extraction of the roots with benzene.

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1. Y.N.Sharma, A.Zaman, and A.R.Kidwai, Tetrahedron, 20, 87 (1964).

2. Y.N.Sharma, R.C.Sharma, A.Zaman and A.R.Kidwai, Naturwiss, 22, 537 (1964).

3. P.Crabbe, P.P.Leming and C.Djerassi, J.Amer.Chem.Soc., 80, 5258 (1958).

The furocoumarin nature of the two compounds was indicated by the presence of three strong absorption maxima in their U.V. spectra typical of the furocoumarins.[4] I.R. spectra of both A and B showed absence of free hydroxy and the presence of 6-membered-α,β-unsaturated lactone carbonyl. Acid catalysed degradation of compound 'A' gave xanthotoxol and a fragrant oil which was identified as geranyl alcohol by VPC and comparison of its 3:5-dinitrobenzoate with an authentic sample. This suggested that the compound was 8-geranoxypsoralen reported earlier by W.L.Stanley and co-workers.[5] However, as the same degradation products were obtained from compound B, showing this also to be 8-geranoxypsoralen; it was suspected that one or the other of these compounds was either a mixture or a geometrical isomer which could only mean that it was neryl instead of geranyl ether. Both compounds gave single fluorescent spots on chromatostrips[6] having identical R_f value, but analytical value of compound 'B' did not agree with those required for 8-geranoxypsoralen.

Comparison of the two products with a sample of 8-geranoxypsoralen kindly placed at our disposal by Dr. W.L. Stanley showed that compound A was identical with this.

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4. D.P.Chakraborty and S.K.Chaudhary, Trans.Bose Res.Inst., 24, 15 (1961).
5. W.L.Stanley and S.H.Vannier, J.Amer.Chem.Soc., 79,3488(1957)
6. J.M.Miller, J.G.Krichner, and G.J.Killer, Anal.Chem., 23, 420 (1951).

The sample sent by him melted here at 53-54°, as against 61-62° reported by him. However, Stanley and co-workers had assigned this structure to their compound merely on the basis of isolation of xanthotoxol on acid degradation and indication of the presence of a C_{10} side chain from the analytical values. They failed further, to isolate any leavulinic aldehyde[7] on ozoholysis. The isolation of two compounds having different melting points but giving identical cleavage product made a re-investigation of the nature of the side chain necessary.

Infrared spectra of the two compounds differed only in the presence of an extra band at 10.6 μ in the spectrum of compound A. The U.V. spectra were also identical. Ozonolysis

Space for I.R.spectra.

of both compounds gave leavulinic aldehyde and acetone which were identified as 2:4-dinitrophenylhydrazone derivatives, thus excluding any difference in the attachment of the side chain to the coumarin residue. This left only the possibility of compound B being either the cis isomer or contaminated with some other impurity.

Recently the nature of the side chain in mycelianamide was re-investigated by Bates and co-workers[8] and the same methods were applied in this case. The stereochemistry of

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7. W.L.Stanley, Proc. Third, Ann.Symp. P.P.G.N.A. Toronto, 79 (1963).

8. R.B.Bates, J.H.Schauble and M.Soucek, Tetrahedron Letters, 25, 1683 (1963).

of the side chain was established by Birch reduction. Compound B was treated with sodium in liquid ammonia, the reaction mixture after complete reduction was diluted with water and heptane. VPC analysis of the product obtained from the organic phase showed peaks attributed to trans-2, 6-dimethyl-2,6-octadiene (methylgeraniolene) and 2-methyl-2-butene, thereby indicating it to be a mixture of imperatorin and 8-geranoxypsoralen. Sufficient material was not available for a similar degradation of compound A, but the presence of a geranyl side chain here also could be established by comparison with synthetic material. The problem of difference in the melting points of these two compounds was settled by comparison of the NMR spectra of the two. A doublet at 4.82τdue to $-O-CH_2-C=C-$ occurs in both C_5 and C_{10} compounds while the absorption at 7.91τcomes from $-C=C-CH_2-CH_2-C=C-$ which occurs only in the C_{10} compound. The integrated intensities of compound B show a 2 to 1 ratio in favour of the $-C-CH_2-C=C$ protons. Thus the side chain must consist of 3 times as much C_5 as C_{10}. This along with the above degradations suggested that it was a mixture of 8-geranoxypsoralen and imperatorin in the ratio of 1:2. This is also in agreement with the analytical values. Repeated attempts to separate the two compounds on chromatostrips on various adsorbents did not succeed. It was found that authentic 8-geranoxypsoralen when mixed with imperatorin in about the same proportion does not separate from this on chromatostrips or on paper chromatograms.

An attempt was made to synthesise 8-geranoxypsoralen using the procedure of Chatterjee and Chaudhary[9] for the synthesis of bergamottin but this did not succeed. It could, however, be synthesised by the method of Schonberg and Sina[10], by refluxing xanthotoxol, geranyl bromide in dry acetone over potassium carbonate. The product after purification gave no depression in mixed melting point with the natural sample.

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9. A.Chatterjee and B.Chaudhary, J.Chem.Soc., 2246 (1961).

10. A.Schonberg and A.Sina, J.Amer.Chem.Soc., 72, 4862 (1950).

EXPERIMENTAL

All ultraiolet spectra were measured in a Beckmann model DU instrument in 95% ethanol. Infrared spectra were taken in a Parkin Elmer Infracord either in chloroform solutions or as mulls in nujol, and NMR spectra were taken on a Varian A-60 spectrometer.

Isolation:

5 gms of the crude mixture of coumarins obtained from the petroleum ether extract of Heracleum candicans roots (80 g.) was chromatographed over acetic acid deactivated alumina, with petroleum ether to give 100 mg. of a low melting semi solid product, sparingly soluble in petroleum ether. Repeated crystallisations from a large excess of this solvent afforded stout needles melting point 53-54°, λ max. 215 mμ(log ϵ 4.51) 248 mμ(log ϵ 4.42) and 298 mμ (log ϵ 4.13).

Analysis: Calculated for $C_{21}H_{24}O_4$: C, 74.09; H, 7.11
Found: C, 74.32; H, 6.73.

Continued elution of the column with petroleum ether benzene (5:1) gave 500 mg. of a solid insoluble in petroleum ether, which crystallised from methanol in needles melting point 82-83° λ max. 217 mμ (log ϵ 4.47), 248 mμ (log ϵ 4.42) and 298 mμ(log ϵ 4.05).

Thin layer chromatography: Chromatostrips for this were prepared according to the procedure of Krichner and Miller using alumina (E.Merk) containing 2% of starch. Both the

products gave one fluorescent spot having identical R_f value, chromatostrips were developed with petroleum ether benzene (7:3).

Acetic acid cleavage: Compound A (2 g.) was treated with glacial acetic acid (2 ml.) in an oil bath at 115-120° for 1½ hours. The reaction mixture was allowed to stand overnight at room temperature when a solid separated out. This was extracted thrice with 50 ml. portions of hexane and the combined hexane layers were washed with water and Na_2CO_3 to remove acetic acid. The hexane extract on evaporation left a sweet smelling oil which was hydrolysed directly with 10% methanolic potash to give 200 mg. of geraniol.

Analysis: Calculated for $C_{10}H_{18}O$: C, 77.86; H, 11.76
Found: C, 78.56; H, 11.92.

The solid left behind after the extraction of the reaction products with hexane was crystallised from ether-hexane to give light yellow crystalline mass, identified as xanthotoxol.

Analysis: Calculated for $C_{11}H_6O_4$: C, 65.35; H, 2.99.
Found: C, 64.98; H, 3.14.

Xanthotoxol acetate: Xanthotoxol (500 mg.) acetic anhydride (5 ml.) and fused sodium acetate (100 mg.) were refluxed for 1 hour. The mixture was cooled, poured over crushed ice and the acetate crystallised from methanol melting point 178°.

Analysis: Calculated for $C_{13}H_8O_5$: C, 63.94; H, 3.30.
Found: C, 64.88; H, 3.51.

Xanthotoxin: Xanthotoxol (500 mg.) in methanol (5 ml.) was treated with an excess of diazomethane in ether and left overnight. The residue was dissolved in benzene and chromatographed over acetic acid deactivated alumina, elution with benzene gave a product melting point 146-147° after crystallisation from benzene petroleum-ether.

Xanthotoxin nitrate: Xanthotoxin (100 mg.) in glacial acetic acid (5 ml.) was treated with nitric acid (sp.gr. 1.42) (.5 ml.). The reaction mixture after standing at room temperature for 2 hours and dilution with water gave a bright yellow solid melting point 236° (Methanol).

3:5-dinitrobenzoate of geraniol: The oil obtained as above (100 mg.) in dry benzene (10 ml.) was treated with a solution of 3:5-dinitrobenzoyl chloride (200 mg.) in dry benzene (10 ml.) with the addition of 2 drops of pyridine and the mixture heated on a water bath for 20 minutes. The reaction mixture, diluted with an excess of ether and the ether-benzene layer washed thoroughly with cold water dried (Na_2SO_4) and the solvent removed in vacuum. Residue crystallised from methanol melting point and mixed melting point 62-63°.

Ozonolysis of compound A: A solution of compound A (500 mg) in glacial acetic acid (20 ml.) was treated with ozonised oxygen for 2 hours. Water (30 ml.) and Zinc dust (100 mg.)

was then added and the mixture warmed on a water bath till it became clear. After dilution with a further quantity of water (70 ml.) the solution was steam distilled and the distillate collected directly in fraction in 2N sulphuric acid solution of 2:4-dinitrophenylhydrazine. The first 10 ml. of distillate yielded a bulky orange precipitate which dissolved completely in hot methyl alcohol. The filtered solution on cooling deposited orange plates of acetone-2:4-dinitrophenylhydrazone melting point and mixed melting point 123-124°. Later fractions of the steam distillate gave a bright yellow derivative which was crystallised from nitro-benzene-alcohol to give a powder identified as laevulinic aldehyde, melting point and mixed melting point 233-234°.

Birch Reduction of Compound B: A three necked 500 ml. round bottom flask was fitted with a dry ice condenser, stirrer and a dropping funnel. Ammonia (75 ml.) was condensed in the flask and sodium metal (3 gm.) was added in small pieces, producing a blue solution. To the vigorously stirring Na/NH_3 solution under an N_2 atmosphere compound B (500 mg.) in 20 ml. of methyl alcohol was added dropwise over ½ hour. The blue colour of Na/NH_3 disappeared during the addition, so some more sodium was added in small pieces to maintain the blue colour for 1 hour. Then heptane (25 ml.) granulated NH_4Cl (10 g.) and water (50 ml.) were added to the refluxing reaction mixture. After separating the heptane layer, the aqueous layer was washed with three 25 ml. portions of heptane. The combined heptane extracts were washed with

water, untill the washings were neutral dried over $MgSO_4$ and subjected to VPC analysis. The VPC analysis showed peaks attributed to 2-methyl-2-butene and trans-2, 6-dimethyl-2, 6-octadiene (methylgeraniolene).

Geranyl bromide: Phosphorus tribromide (12 gm.) in petroleum ether (40-60°) (10 ml.) was added dropwise during one hour at -7° to geraniol (15 g.) in petroleum ether 29 ml. and pyridine (2.5 ml.) with constant stirring. The reaction mixture was further stirred for 20 minutes at -7°, poured over crushed ice, stirred for 15 minutes and then treated with petroleum ether (100 ml.). The petroleum ether layer was separated, washed twice with water and once with dilute solution of sodium bicarbonate, dried (Na_2SO_4) and solvent evaporated off. The residue was distilled at 102-103° at 3 mm. pressure.

Condensation of xanthotoxol and geranyl bromide: A mixture of xanthotoxol (0.2 g.), anhydrous potassium carbonate (3 g.), anhydrous acetone (50 ml.) and geranyl bromide (2 ml.) was refluxed for 36 hours. Acetone solution was filtered and the residue washed five times with 20 ml. portions of dry acetone. The acetone washings were combined with the main bulk and solvent removed. The residue dissolved in a minimum amount of dry benzene and chromatographed over acetic acid deactivated alumina, elution with petroleum ether gave a viscous mass, which crystallised from petroleum

ether in needles melting point and mixed melting point 53-54°. The IR spectra of natural and synthetic products were superimposable.

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